COLUMBIA UNIVERSITY

Association of Sleep Duration and Quality with Activation of Two Neuroendocrine Systems: Hypothalamic-Pituitary-Adrenal Axis and Sympathetic Nervous System. The Multi-Ethnic Study of Atherosclerosis (MESA)

Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy under the Executive Committee of the Graduate School of Arts and Sciences

Castro-Diehl, Olga Cecilia

12/10/2015

Committee:
Steven Shea, Chair
Judith S. Jacobson, Sponsor
Pam Factor-Litvak, Second Reader
Ana V. Diez-Roux, Drexel University
Bruce S. McEwen, Rockefeller University

ABSTRACT

Association of Sleep Duration and Sleep Quality with Activation of Two Neuroendocrine Systems: Hypothalamic-Pituitary-Adrenal Axis and Sympathetic Nervous System. The Multi-Ethnic Study of Atherosclerosis (MESA)

Olga Cecilia Castro-Diehl

Many studies have shown that short sleep duration and/or poor sleep quality is associated with increasing rates of cardiovascular (CVD) mortality and morbidity. One hypothesized explanation for this association has been that sleep loss is a type of chronic stress that induces dysregulation of biological systems that ultimately increase the risk of CVD. One biological system that has been thought to link sleep loss and CVD is the hypothalamus-pituitary-adrenal (HPA) axis. A number of studies using small or convenience samples have addressed the effects of sleep deprivation on cortisol. Only a few studies have examined the association of habitual short sleep duration and/or poor sleep quality with changes in the diurnal cortisol in population based-samples; those studies vary in their methodology and in findings.

Another biological system that has been thought to link sleep loss and CVD is the autonomic nervous system (ANS), through overactivation of the sympathetic nervous system (SNS) and/or probably a withdrawal of the parasympathetic nervous system. Experimental studies have shown an association between the sleep stages and markers of the sympathetic system. However, very few studies of habitual sleep duration/sleep quality and ANS markers have been conducted. Even fewer studies have examined the association of habitual sleep duration and/or sleep quality and ANS responses to a stress challenge in a population-based sample. The findings again have been inconsistent probably due to the use of different methodology and different samples.

This dissertation used measures of salivary diurnal cortisol as well as cortisol responses to a stress challenge protocol to assess the relationship of habitual sleep duration and/or sleep quality with diurnal cortisol profile in natural conditions and in response to a stress challenge protocol in a laboratory setting. Diurnal cortisol was assessed from up to 16 samples of salivary cortisol for two days. Cortisol responses to a stress challenge were assessed from four salivary samples taken during the stress challenge that was

performed in a different day than the diurnal cortisol collection. To examine the relationship of habitual sleep duration and/or sleep quality and markers of the ANS, this dissertation used continuous cardiovascular measures (heart rate and heart rate variability) and four salivary amylase samples obtained during the stress challenge. The stress challenge included mental stress and orthostatic stress.

Sleep duration and sleep efficiency (an objective measure of sleep quality) were assessed from 7-day actigraphy and sleep diaries. Insomnia symptoms (a subjective measure of sleep quality) were also assessed using a questionnaire that included the Women's Health Initiative Insomnia rating scale (WHIIRS).

We used mixed models so as to account for the repeated measures of diurnal salivary cortisol levels as well as the responses (reactivity and recovery) to the stress challenge tests.

Chapter 1 presents an introduction to this dissertation discussing the relationship between short sleep duration and/or poor sleep quality and CVD morbidity and mortality.

Chapter 2 presents a systematic literature review of studies of the association between habitual sleep duration and/or sleep efficiency and markers of neuro-endocrine systems: HPA and ANS. These are plausible mechanisms that link short and/or poor sleep to CVD morbidity and mortality.

Chapter 3 presents our analyses of the relationship between short sleep duration and/or poor sleep quality and features of the diurnal cortisol. We hypothesized that those participants whose slept < 6 hours per night or whose sleep efficiency was < 85% would have higher cortisol levels on awakening, flatter cortisol awakening responses (CAR), and higher evening cortisol levels than participants who slept longer or slept better. We found that short sleepers had higher evening cortisol than the longer sleepers and that this association persisted after the adjustment for several known confounders.

In chapter 4, we examined how the same groups of participants responded in terms of hormones (cortisol and amylase) and cardiovascular indices (heart rate (HR) and HR variability (HRV)) to a stress challenge test. We hypothesized that those participants who slept for a shorter time or whose sleep was of poorer quality would have more exaggerated responses to and less recovery from a stress challenge test than participants who slept longer or slept better. We found that participants with insomnia had exaggerated high frequency-HRV (HF-HRV) orthostatic reactivity. In an extended analysis, we found that

participants who slept less than 7 hours/night had exaggerated heart rate reactivity to a mental stress test compared to participants who slept 7 or more hours/night, but this association was attenuated after adjustment for naps. Paradoxically, we also found that participants who slept less than 7 hours had higher HF-HRV recovery from mental stress compared to longer sleepers (≥7 hours). Short sleep duration or low sleep efficiency was not associated with cortisol or amylase responses to the stress challenge protocol.

These findings suggest that sustained high evening cortisol levels and cardiovascular responses to a stress challenge may be among the mechanisms linking short/poor sleep and CV disease.

TABLE OF CONTENTS

LIST OF TABLES ..... **VIII**

TABLE OF FIGURES ..... **IX**

ACKNOWLEDGEMENTS ..... **X**

DEDICATION ..... **XI**

1. CHAPTER 1. INTRODUCTION **1**

SLEEP DURATION AND SLEEP EFFICIENCY, THE HYPOTHALAMIC-PITUITARY ADRENOCORTICAL (HPA) AXIS AND THE AUTONOMIC NERVOUS SYSTEM (ANS) IN CARDIOVASCULAR DISEASE (CVD) **1**

BACKGROUND 1
PHYSIOLOGY OF THE NEUROENDOCRINE STRESS SYSTEMS 3
The Hypothalamic-Pituitary-Adrenal (HPA) Axis *4*
The Autonomic Nervous System (ANS) *5*
SUMMARY 7
STATEMENT OF PURPOSE 7

2. CHAPTER 2. A SYSTEMATIC REVIEW OF THE LITERATURE **10**

THE RELATIONSHIP BETWEEN SLEEP DURATION AND/OR SLEEP EFFICIENCY AND THE HYPOTHALAMIC-PITUITARY ADRENOCORTICAL (HPA) AXIS AND THE AUTONOMIC NERVOUS SYSTEM (ANS) **10**

BACKGROUND 10
METHODS 11
Sleep duration/sleep efficiency and diurnal cortisol *12*
Sleep duration/sleep efficiency and cardiovascular responses to a stress challenge test *13*
Sleep duration/sleep efficiency and hormonal responses to stress *13*
RESULTS 14
Sleep duration/sleep efficiency and diurnal cortisol *14*
Sleep duration/sleep efficiency and cardiovascular responses to a stress challenge *15*
Sleep duration/sleep efficiency and hormonal responses to a stress challenge *16*
DISCUSSION 16
CONCLUSIONS 19

3. CHAPTER 3. ASSSOCIATIONS OF SLEEP DURATION AND QUALITY WITH ACTIVATION OF THE HYPOTHALAMIC-PITUITARY ADRENOCORTICAL (HPA) AXIS **28**

ABSTRACT 28
INTRODUCTION 29
METHODS 31
Participants *31*
Outcome Variables *33*
Exposure Variables *33*
Covariates *34*
Analysis *35*
RESULTS 39
Sleep Duration *39*

Sleep efficiency *40*
Insomnia *40*
DISCUSSION 42

4. CHAPTER 4. SLEEP DURATION AND QUALITY AND HORMONAL AND CARDIOVASCULAR RESPONSES TO A STRESS CHALLENGE PROTOCOL 56

ABSTRACT 56
INTRODUCTION 57
Cardiovascular responses to the mental stress challenge test *57*
Hormonal responses to the mental stress challenge test *58*
METHODS 59
Participants *59*
Sleep Protocol *61*
Stress Challenge Protocol (Mental and Orthostatic Stress) *61*
Covariates *64*
Statistical Analyses *64*
RESULTS 66
Heart rate (HR) and HF-HRV responses to the stress challenge *67*
Amylase and cortisol responses to the stress challenge test *68*
DISCUSSION 68

5. CHAPTER 5. CONCLUSIONS 81

SLEEP DURATION AND SLEEP EFFICIENCY, THE HYPOTHALAMIC-PITUITARY ADRENOCORTICAL AXIS (HPA) AND THE AUTONOMIC NERVOUS SYSTEM (ANS). 81

AIMS 81
SUMMARY OF RESULTS 81
Short sleep duration associated with evening cortisol *83*
Responses to stress challenge (acute stress) *84*
LIMITATIONS 85
IMPLICATIONS 86
CONCLUSIONS 86

REFERENCES 88

APPENDICES 97

LIST OF SUPPLEMENTAL TABLES 98

LIST OF SUPPLEMENTAL FIGURES 99

LIST OF TABLES

Table 2-1. Quasi-systematic literature of epidemiological studies examining associations between short sleep duration and/or poor sleep quality and diurnal cortisol 20

Table 2-2. Quasi-systematic literature of studies examining associations between short sleep duration and/or poor sleep quality and cardiovascular responses to a stress challenge 24

Table 2-3. Quasi-systematic literature of studies examining associations between short sleep duration and/or poor sleep quality and hormonal responses to a stress challenge test 25

Table 3-1 Characteristics of participants (n= 600) by sleep duration and sleep efficiency, MESA Study (2010-2012) 47

Table 3-2. Characteristics of study participants by insomnia symptoms (n= 591) and overall, MESA Study (2010-2012) 49

Table 3-3.Percent differences (95% confidence intervals) in features of the daily cortisol (wake-up, CAR, early decline and late decline) and summary measures of cortisol (wake-to-bed slope and AUC) associated with sleep duration and sleep efficiency 51

Table 3-4. Percent differences (95% C.I.) in features of the daily cortisol and summary measures of cortisol associated with insomnia and stratified by short and longer sleepers 52

Table 3-5. Percent differences (95% confidence intervals) in features of the daily cortisol and summary measures of cortisol associated with sleep duration specified as dichotomized and continuous stratified by insomnia symptoms 53

Table 4-1. Characteristics of participants (n= 527) by sleep duration, sleep efficiency and insomnia symptoms, MESA Study (2010-2012) 73

Table 4-2. Mean differences in log-transformed heart rate (log (beats/min)) at baseline and mean differences in responses to challenge by sleep duration, sleep efficiency and insomnia symptoms (N = 3450 observations, 527 persons) 74

Table 4-3. Mean differences in log-transformed HF-HRV (log ($msec^2$)) at baseline and mean differences in responses to challenge by sleep duration, sleep efficiency and insomnia symptoms (N = 3450 observations, 527 persons) 75

Table 4-4. Mean differences in log transformed amylase ((log (U/mL)) at baseline and mean differences in responses to challenge by sleep duration, sleep efficiency and insomnia symptoms (N=1736 observations, 454 persons) 76

Table 4-5. Mean differences in log transformed cortisol ((log (nmol/L)) at baseline and mean differences in responses to challenge by sleep duration, sleep efficiency and insomnia symptoms (N=2085 observations, 532 persons) 77

TABLE OF FIGURES

Figure 2-1. Flow chart of articles selected for sleep duration/sleep efficiency and diurnal cortisol 26

Figure 2-2. Flow Chart of articles selected for sleep duration/sleep efficiency and cardiovascular responses to a stress challenge.....27

Figure 3-1. Features of the diurnal cortisol profile 54

Figure 3-2. LOESS plot of the cortisol daily profile for male and women.....55

Figure 4-1 Flow Chart of Exclusion Criteria for Participants with Cardiovascular Measures.....78

Figure 4-2 Stress challenge protocol: measures of heart rate (HR), HR variability (HRV) and salivary samples (amylase and cortisol) 79

Figure 4-3 Mean Heart Rate (HR), HF-HRV and amylase for the whole population during the MESA Stress protocol.....80

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my sponsor, Dr. Judith Jacobson, for her continuous support during all my years in the doctoral program, for her patience and guidance during courses and dissertation. I am so grateful for our weekly discussion and conversational meetings.

I would like to thank Dr. Steven Shea for being a wonderful mentor. His encouragement and questions and comments have been very constructive during my student life. He has also been my role model for hard work. He gave me the opportunity to coordinate the MESA study all these years, and placed great confidence in my work, for which I am very grateful.

My immense gratitude also goes to Dr. Ana Diez Roux, who allowed me to work in the collection and then analyses of the Stress data and provided me an opportunity to join her discussion group, from which I have learned so valuable knowledge, and the tools to analyze complex data. Without her support it would not be possible to conduct this dissertation.

I would like to thank the other members of my committee members, Dr. Pam Factor-Litvak and Dr. Bruce McEwen, whose work have been inspirational for me.

A special thanks to my family members and friends, who at the distance want the best things for me. I also thank my MESA co-workers and friends, Vijay and Analicia, for their patient in listening to me each time I found something interesting during the process of my dissertation. They shared with me the fascination of the news.

Last but not the least; I would specially like to thank my lovely mother, Cecilia, and my lovely husband, Karl, for their spiritual support throughout writing this dissertation. They are always with me at every moment with their unconditional love.

DEDICATION

To the loving memory of my father.

1. CHAPTER 1. INTRODUCTION

SLEEP DURATION AND SLEEP EFFICIENCY, THE HYPOTHALAMIC-PITUITARY ADRENOCORTICAL (HPA) AXIS AND THE AUTONOMIC NERVOUS SYSTEM (ANS) IN CARDIOVASCULAR DISEASE (CVD)

Background

There is an increasing awareness of the association between sleep duration and quality and cardiovascular disease (CVD). This association has been described as U-shaped as both the highest and lowest levels of sleep duration are associated with poorer health outcomes. In the early 1960s, the American Cancer Society,[1] interviewed and then followed more than one million Americans and found that men who had reported sleeping either ≤5 hours or ≥10 hours per night had higher death rates than those who reported sleeping 6- 9 hours per night. Further follow up[2] found that, in men, the mortality ratio for coronary heart disease (CHD) was higher among those who slept ≤4 hours per night (2.08) and among those who slept ≥10 hours per night (1.51) than among those who slept 7- 7.9 hours; in women, the CHD mortality ratio was also higher among those who slept ≤4 hours per night (1.10) and among those who slept ≥10 hours per night (1.67) than among those who slept 7- 7.9 hours. At the beginning of the 21st century, people in the United States reported sleeping, on the average, 7½ hours per night, 1½ hours less than in the previous century.[3] The 2009 Behavioral Risk Factor Surveillance System reported that among 74,571 adult respondents from 12 states, 35% of respondents sleep less than seven hours per day.[4] Subsequent epidemiological studies have also found that people who reported either short or long sleep duration were at higher risk of all-cause mortality than people with sleep duration in the middle range.[5,6]

The association between sleep duration and health, described as following a U-shape distribution (in which people who are in the extremes have poorer health outcomes than people who are in the middle), has been increasingly reported in subsequent studies. A pooled analysis of 16 published articles about 27 cohorts,[7] showed that people who slept <7 hours had a 12% greater risk, and people who slept >8 hours a 30% greater risk of all-cause mortality than people who slept 7- 8 hours per night.

Cardiovascular disease (CVD) and CVD risk factors have been linked to both short and long sleep duration in women.[8] In a meta-analysis of fifteen studies and 24 cohorts,[9] short sleep duration was

associated with greater risk of CHD (RR 1.48, 95% CI 1.22–1.80) and stroke (RR 1.15, 95% CI 1.0-1.31) and long sleep duration was also associated with greater risk of CHD (RR 1.38, 95% CI 1.15-1.66) and stroke (RR 1.65, 95% CI 1.45-1.87) than the middle range of sleep duration. Studies have also found U-shaped associations between sleep duration and obesity,[10-12] hypertension,[13] and diabetes and insulin resistance.[14] However, a 12-year longitudinal sleep study from Netherlands, the Monitoring Project on Risk Factors and Chronic Diseases in the Netherlands (MORGEN) Study,[15] which followed more than 23,000 participants, linking their subjective measures of sleep duration and quality with national health registries, showed that short sleepers had higher risk of CVD (HR 1.15, 95% CI 1.00-1.32) and CHD (HR 1.23, 95%CI 1.04-1.45) than normal sleepers whereas long sleepers did not have higher risk for CVD, but lower risk of CHD.

The mechanisms by which both short and long sleep duration may lead to CVD and related outcomes are not well understood and may differ. Whereas several mechanisms have been proposed to account for the association of short sleep duration with CVD, long sleep duration has been proposed as an independent risk factor for mortality[16] and as a marker of poor physical and mental health that leads to mortality.[17] Because short sleep duration has been most often linked to CVD outcomes than long sleep duration, this dissertation concentrates on short sleep duration.

Short sleep duration has been linked to clinical CVD[15,18] as well as subclinical CVD, as indexed by coronary artery calcium[19] and carotid intima-media thickness.[20] Short sleep duration has also been associated with CVD risk factors including hypertension,[13,21,22] insulin resistance,[23] obesity,[24] and diabetes.[25]

In addition, poor sleep quality, assessed either subjectively through self-report measures of insomnia, or through objective measurements of low sleep efficiency on actigraphy or reduced time in slow wave sleep (SWS) on polysomnography, has also been associated with adverse CVD outcomes. For example, insomnia has been associated with increased rates of myocardial infarction,[26] and self-reported sleep quality on the Pittsburgh Sleep Quality Index has been linked to the metabolic syndrome.[27] In a cohort of older, mostly white, men, reduced time in SWS has been associated with hypertension[28] and

obesity. [29] Low sleep efficiency has also been associated with elevated blood pressure among healthy adolescents.[30]

In the MORGEN study,[15] participants with both short sleep duration and poor sleep quality had a 63% higher risk of CVD than those with normal sleep duration and good sleep quality. In particular, it has been suggested that short sleep duration is more strongly associated with cardiometabolic outcomes when it is accompanied by insomnia symptoms than when it is not.[31-33]

Two mechanisms that are thought to link short sleep duration and/or poor sleep quality with CVD outcomes are dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis leading to alterations in diurnal cortisol profile, and dysregulation of the autonomic nervous system (ANS) in which its sympathetic branch may be overactivated or its parasympathetic branch may not modulate the excessive activity of the sympathetic branch. These systems are also involved in the response to stress, and sleep deprivation is considered a type of chronic stressor.[34,35] Alterations of the HPA and SNS are not the only mechanisms by which short sleep duration and/or poor sleep quality may lead to higher risk of CVD and related outcomes. Whereas the HPA and SNS interact and reinforce each other during emotional stress, cortisol and catecholamines also initiate an inflammatory response, and acute and chronic stress can trigger an inflammatory response initiated centrally in the brain.[36] Both inflammatory and immunologic biomarkers may also be found in the pathway between short/poor sleep and CVD (Supplemental Figure A-1). Interactions between the HPA and SNS, along with inflammatory biomarkers and metabolic factors, may play a key role in the association of sleep with CVD and related outcomes.[34,37]

Physiology of the neuroendocrine stress systems

The physiological response to stress involves release of adrenocorticotropic hormone (ACTH) from the anterior pituitary, cortisol from the adrenal cortex, epinephrine from the adrenal medulla, and norepinephrine from the sympathetic nerves.[38] The production of catecholamines (epinephrine and norepinephrine) is regulated by the sympathetic nervous system (SNS). The HPA axis and the SNS interact and reinforce each other during emotional stress, in which corticotropin-releasing hormone (CRH) stimulates the synthesis and secretion of ACTH, and norepinephrine potentiates these effects.[39]

Glucocorticoids also regulate catecholamine biosynthesis in the adrenal medulla, and catecholamines stimulate ACTH release from the anterior pituitary gland.[38]

The Hypothalamic-Pituitary-Adrenal (HPA) Axis

The HPA axis and CVD

Dysfunction of the HPA, as indexed by increases of urinary cortisol levels or alterations in diurnal cortisol profile, predicts CVD mortality. For example, a cohort study of more than 800 elderly people found that those with higher urinary cortisol levels had higher risks of CVD mortality.[40] Alteration in diurnal salivary cortisol profile has also been associated with cardiovascular-related mortality and with subclinical CVD. A large epidemiological study of more than 4000 public workers[41] showed that flatter slope in cortisol levels was associated with higher risk of cardiovascular mortality. Flatter diurnal salivary cortisol slopes have also been associated with the presence of coronary artery calcification.[42] Higher diurnal total salivary cortisol levels have also been associated with higher numbers of plaques on carotid arteries.[43] These studies suggest a role of cortisol in atherosclerosis.

Cortisol responses to an acute psychological stress in laboratory have also been studied as proxies for the mechanism by which mental stress is associated with CVD. Exaggerated cortisol responses to a mental stress have predicted subclinical CVD and CVD risk factors, such as coronary artery calcification[44] and its progression.[45] Cortisol reactivity (cortisol response to i.e. a mental stress in laboratory settings) has also been associated with greater risk of hypertension.[46]

Sleep and the HPA axis

Experimental and observational studies of the relationship of sleep duration and/or sleep quality with the HPA axis have shown inconsistent results, maybe in part because of differences in sample and measurement approaches. Experimental studies in which volunteers underwent total or partial sleep deprivation have been limited to small samples and specific groups such as only women or only men. For example, in a study of 33 young men,[47] evening plasma cortisol levels were higher after a night of sleep deprivation than after a night of normal sleep. Similarly, in another study of 11 healthy young men,[48] evening salivary cortisol levels were higher after a night of partial sleep deprivation than after a night of normal sleep. Another study of 17 healthy young women[49] found reduced salivary morning cortisol levels

30 minutes after awakening, elevated afternoon/evening cortisol and slow decline of cortisol levels from morning to evening following a single night of sleep restriction. However, in another study of healthy young men,[50] diurnal plasma cortisol levels were lower after a night of sleep deprivation than after a night without sleep deprivation. Similarly, in a study of 13 young women who were exposed to sleep disturbance during three consecutive nights,[51] the cortisol awakening response (CAR) after disturbed nights was not different from that after undisturbed nights.

Epidemiological studies of the relationship between sleep duration and/or sleep quality and cortisol levels are few and they also have few consistent results as we will discuss in the next chapter.

Markers of the Hypothalamic-Pituitary-Adrenal (HPA) Axis

Free cortisol (the cortisol that is not bound to glucocorticoid receptors) is a marker of the HPA axis that can be detected in blood, urine and saliva. Salivary cortisol sampling is most convenient for epidemiological studies because it is a non-invasive marker of the HPA function and it is feasible to collect it for several times a day for more than one day. Cortisol levels in plasma and saliva rise and fall throughout the 24-hour sleep-wake cycle, reaching a nadir 2-4 hours before the onset of sleep.[52] They rise about 50-75% within the first 30 minutes after awakening; this pattern is called the cortisol awakening response (CAR).[53] In the next 2-3 hours after awakening, cortisol levels decline sharply; during the rest of the day, levels continue to fall but more gradually.[52,54]

Cortisol levels also respond, of course, to stress. In the laboratory, cortisol responses to mental stress tests (e.g., the Trier Social Test (TSST),[55] the color-word conflict task,[56] and the arithmetic task[57] have been investigated as proxies for the effect that acute stress has on the HPA axis function.

The Autonomic Nervous System (ANS)

The ANS and CVD

Epidemiological studies have shown that increased heart rate (the result of the joint influence of sympathetic and parasympathetic nervous system on the sino-atrial node), both at rest and in response to exercise, can predict cardiovascular mortality.[58,59] Studies of cardiovascular reactivity to stressors have evaluated the activation of the autonomic nervous system in response to stress challenges as well as the effects of stress responsivity on the cardiovascular system. For example, in the CARDIA study, blood

pressure reactivity during psychological and physical stress predicted hypertension 13 years later among black and white men and women.[60] In the same group of participants, blood pressure reactivity to mental stress (video game) predicted the presence of coronary calcium.[42]

Sleep and the ANS

Experimental studies have consistently showed that sleep regulates sympathetic activity. Circulating levels of norepinephrine and epinephrine have been studied in different stages of sleep, and periods of wakefulness before and after sleep. Plasma concentrations of both catecholamines are lower during sleep than during the periods of wakefulness (before, during or after sleep).[61] In experimental studies, plasma catecholamine levels were higher during the hours of the nights that subjects were awakened than when they were not, and the lowest level of plasma norepinephrine was during the slow wave sleep (SWS).[62] Similarly, heart rate and blood pressure, which are normally influenced by the SNS, were lower during stage 2 and SWS than in other sleep stages or wakefulness.[63] Insomnia, a disorder characterized by perceived difficulties in initiating or maintaining sleep, a state of "hyper-arousal,"[64] has been associated with abnormalities in autonomic nervous system activity.[65,66]

Cardiovascular reactivity to stressors has been examined in a few experimental studies; most of them with small samples of healthy volunteers who underwent sleep deprivation before the mental stress challenge. Some have found associations between sleep deprivation and autonomic alterations,[67,68] but others have not.[69]

Markers of the Autonomic Nervous System (ANS)

Catecholamine levels in plasma and urine reflect activation of the SNS.[38] Although techniques such as microneurographic measurement of muscle sympathetic nerve activity and organ specific norepinephrine spillover[70] are more specific and reliable, they are not useful for large population studies. Measures that are commonly used in epidemiological studies include blood pressure, heart rate (HR), blood pressure and HR variability, and most recently salivary amylase.[71] Sympathetic activation results in the secretion of parotid saliva with high concentrations of amylase and low flow rates, whereas parasympathetic activation results in saliva with low concentrations of amylase and high flow rates.[72] Studies have found associations between salivary alpha amylase (sAA) release after psychological and

physical stress and indicators of sympathetic activity, such as plasma norepinephrine[73] and shortened cardiac pre-ejection period.[74] sAA has been proposed as a useful and novel stress marker[75] because it responds to stress and it is simple to collect.

Summary

The relationship between alterations in sleep and both the HPA and the SNS have been examined in many experimental studies in which small samples of healthy participants in their 20s, only women, or only men were exposed to acute sleep deprivation or interruption. Abnormal sleep patterns, such as short sleep duration and poor sleep quality and their association with these two stress systems have not been studied extensively in community-based samples under habitual sleep conditions.

Statement of Purpose

Although sleep duration and quality are associated with cardiovascular disease and related outcomes, the mechanisms of this association have not been determined. Several mechanisms that are thought to be involved in this association include dysfunction of the HPA, dysregulation of the autonomic nervous system (in which the sympathetic and parasympathetic nervous system are unbalanced), and increases in inflammatory biomarkers and metabolic factors. The interactions of the hormonal and inflammatory systems along with the metabolic factors may play an important role (Supplemental Figure A-1) in the association of short/poor sleep and poor health, specifically CVD. The INTERHEART study, a case-control study about potentially modifiable risk factors for myocardial infarction (MI) conducted in 52 countries, reported that nine risk factors (including smoking, lack of exercise and lack of consumption of fruits and vegetables, as well as, hypertension, obesity and diabetes among others) accounted for about 90% of the risk for acute MI.[76] The population attributable risk (99% CI) for the nine potentially modifiable risk factors was 88% (84-91) for older adults both men and women. This finding suggests that approximately 10% of the risk of acute MI remains to be explained by other potentially modifiable risk factors. Perhaps, short sleep duration and/or poor sleep quality could also enter in the list of preventable MI risk factors in the near future.

The purpose of this study was to examine some of the possible mechanisms involved in the relationship of short sleep duration and/or poor sleep quality with CVD in a sample of participants from

the Multi-ethnic study of atherosclerosis, the MESA study. Data from two MESA ancillary studies (Sleep and Stress) were linked in order to study these associations. We predicted that participants who have either short sleep duration or poor sleep quality or both would have alterations in both HPA and SNS functions. We also assessed the role of race and socioeconomic status as possible moderators of this association. Studies have suggested that African-Americans had poorer health-related quality of life than Whites as a result of frequent snoring, insomnia symptoms and excessive daytime sleepiness.[77] In the MESA Study, African-Americans had 5 times, Asian-Chinese had 2.3 times, and Hispanics had 1.8 times higher odds of short sleep duration compared to Whites.[78] The sleep deficits in this group maybe the result of socioeconomic status and social support differences across these racial/ethnic groups.

We studied sleep and markers of the HPA and SNS in an ethnically mixed study population. MESA is a large population-based multiethnic cohort study with a variety of measures at several time points. The MESA Sleep Study obtained measures of habitual sleep duration and sleep quality through direct monitoring with both seven-day actigraphy and one-night home polysomnography (PSG). The MESA Stress Study collected up to 16 measures of cortisol over 2 days and four salivary cortisol samples during a stress challenge. It also collected measures of sympathetic/parasympathetic activation (heart rate, blood pressure, heart rate variability and salivary alpha-amylase) under resting conditions and in response to a standardized stress challenge protocol. These two ancillary studies were conducted in largely overlapping subsets of the MESA cohort at Exam 5.

We investigated the associations between chronic, habitual short sleep duration and poor sleep quality and two neuro-endocrine (HPA and SNS) systems in these MESA datasets.

The specific aims of this dissertation proposal were:

Aim 1: To examine the associations of sleep duration and sleep quality with diurnal patterns in salivary cortisol using data from 7-day actigraphy-based measures of sleep duration and sleep quality and ≤16 measurements of salivary cortisol.

Hypothesis 1a: Participants with short sleep duration will have a lower cortisol awakening response (CAR), and shallower cortisol decline during the day than participants with longer sleep duration, before

and after control for age, gender, race/ethnicity, socioeconomic, psychosocial and behavioral factors, medications, apnea, and sleep quality.

Hypothesis 1b: Participants with poor sleep quality will have a lower cortisol awakening response (CAR), and shallower cortisol decline during the day than participants with better sleep quality, before and after control for age, gender, race/ethnicity, socioeconomic, psychosocial and behavioral factors, medications, apnea, and sleep duration.

Aim 2: To examine the association of sleep duration and sleep quality with measures of hormonal and cardiovascular markers, in response to and recovery from a standardized stress challenge protocol.

Hypothesis 2a: Participants whose sleep was short or of poor quality would have higher level heart rate (HR) and higher value of amylase and cortisol at baseline and lower level high-frequency heart rate variability (HF-HRV) at baseline than individuals who slept longer or better.

Hypothesis 2b: Participants whose sleep was short or of poor quality would have greater HR and greater amylase and cortisol reactivity and reduced HF-HRV reactivity to mental and orthostatic stress challenges than individuals who slept longer or better.

Hypothesis 2c: Participants whose sleep was short or of poor quality would have slower HR, HF-HRV, and slower amylase and cortisol recovery from stress to baseline than individuals who slept longer or better.

Aim 3: To test for interaction in the association between short sleep duration and/or poor sleep quality and cortisol with gender, race/ethnicity, and SES.

Hypothesis 3a: This relation differs by race/ethnicity, with stronger association among African Americans and Hispanics compared to whites.

Hypothesis 3b: This relation differs by SES, with stronger association among low SES compared to high SES

2. CHAPTER 2. A SYSTEMATIC REVIEW OF THE LITERATURE

THE RELATIONSHIP BETWEEN SLEEP DURATION AND/OR SLEEP EFFICIENCY AND THE HYPOTHALAMIC-PITUITARY ADRENOCORTICAL (HPA) AXIS AND THE AUTONOMIC NERVOUS SYSTEM (ANS)

Background

Numerous studies have shown associations between short sleep duration and/or poor sleep quality and cardiovascular (CVD) outcomes. Among the mechanisms that may be involved in these associations are dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis and/or overactivation of the sympathetic nervous system (SNS).

Most studies of the associations of short and/or poor sleep with dysfunction of the HPA and hyperactivation of the SNS have been conducted under experimental conditions of acute curtailment of sleep or sleep interruptions. Apparently, few epidemiological studies have examined these associations, and these studies have used different methodologies making comparability across them difficult. For example, some studies have used urinary cortisol,[79] whereas others have used salivary cortisol.[80,81] The protocol of salivary cortisol collection has varied in timing of sample collection during the day, and number of samples collected per day, as well as, number of days of collection.

The experimental studies dealing with the effects of acute sleep deprivation on the HPA and SNS markers have been conducted in specific populations: healthy young adults, only men[48] or only women.[49] The effects that sleep deprivation produces in the neuroendocrine systems of these study participants may differ from the effects of habitual chronic sleep loss in an older population like MESA participants. A few studies to date have focused on habitual short sleep duration and/or poor sleep quality and diurnal cortisol in community-based samples.[79-81] Most of these studies have also used only subjective measures of sleep,[80,81] which may not correlate very well with objective measures of sleep; for example subjective sleep duration may be overestimated.[82,83]

In addition, only a few studies have evaluated how short sleep duration and/or poor sleep quality affects the cardiovascular and hormonal responses to a stress challenge test. Most of these studies have been done in sleep laboratories and have used different types of challenges. Some studies have used

mental stress tests, such as the Trier social test,[55] while others used physical stress tests, such as the isometric handgrip exercise.[84] The response to stress may depend on the type of task participants perform; therefore studies in which participants perform more than one task may be preferable to those involving a single task.[85] The cardiovascular outcomes assessed also vary and most studies have used more than one cardiovascular measure, i.e. hear rate, heart rate variability or blood pressure. Using several measures of cardiovascular response in a given study may also help to increase consistency in the results.[86]

The goal of this systematic review was to determine if short sleep duration and/or poor sleep quality may be associated with alterations of the diurnal cortisol profile and of the cardiovascular and hormonal responses to a stress challenge test in epidemiological studies as experimental studies have found. We conducted three systematic reviews to study: (1) sleep duration and/or sleep efficiency and diurnal cortisol, (2) sleep duration and/or sleep efficiency and cardiovascular responses to a stress challenge, and (3) sleep duration and/or sleep efficiency and hormonal responses to a stress challenge. We included only studies in which the exposure was habitual sleep; sleep in the participants' current environment. Studies of deprivation of sleep were not included because they are usually conducted in experimental conditions rather than in everyday life. In addition, sleep deprivation studies usually recruit only young and healthy volunteers probably because they are more readily available. We included sleep deprivation only if we did not find any study in any of the categories above. Although the stress challenge is conducted in laboratory settings, it is a method of measuring the outcome, not the exposure.

Methods

Articles in the systematic review for the three subsections described below were identified through two databases: OvidMedline and Web of Science. The inclusion criteria were: 1) original research studies published in scientific journals on paper or online between 1995 and 2015, 2) English language, 3) "healthy" adult humans, 4) alterations of the diurnal cortisol profile and/or cardiovascular and hormonal responses to a stress challenge test were the primary outcomes, rather than secondary outcomes or covariates, 5) sleep was measured under "normal "habitual conditions (in the home environment), 6) sleep was objectively measured or subjectively measured using scales already validated, and 7) the

analyses were at least correlational and controlled for the demographic variables that are known confounders of the relationship between sleep duration/sleep efficiency and cortisol. We excluded studies 1) that involved manipulation of sleep duration or quality such as sleep deprivation or sleep interruption, 2) that examined short sleep duration and or poor sleep quality associated with burnout or fatigue, and 3) that involved a specific health condition such as cancer or Cushing syndrome.

Sleep duration/sleep efficiency and diurnal cortisol

The search terms included "salivary cortisol" or "saliva cortisol" or "urinary cortisol" or "diurnal cortisol" or "diurnal cortisol profile" or "diurnal cortisol pattern" and "sleep duration" or "sleep efficiency" or "sleep quality" or "insomnia" or "difficulty falling sleep." We filtered our search to adult humans, English language and publications only in the last 20 years.

We found 365 articles. These articles were imported to Covidence©, a free online software useful for systematic reviews, in which we conducted the qualitative review through the following steps (see also Figure 2-1):

1. We screened the titles and abstracts for relevance (206 articles were irrelevant and 6 duplicate)
2. We screened the full text and excluded134 articles due to:
    a. Wrong study population (e.g., pediatric population or pregnant women) (N=29),
    b. Experimental sleep studies (e.g., sleep deprivation) (N=35)
    c. Wrong setting, such as burnout and fatigue (N=25),
    d. Health problems such as cancer, depression, obesity, Cushing, and PTSD (N=15)
    e. Articles not published in journals (e.g., conference abstracts) (N=12)
    f. Cortisol as secondary outcome or covariate (N=8) because the cortisol outcomes were described but not analyzed
    g. Only very simple analysis, such as t-test (N=1);
    h. Shift workers (N=6)
    i. Non-original research, such as reviews (N=3)
3. We systematically extracted information from the 19 articles included, specifically: sample size, characteristics of the population studied, study design, cortisol sampling and derived cortisol variables,

statistical methods applied, sleep measures, and major significant findings. This information is shown in Table 2-1.

Sleep duration/sleep efficiency and cardiovascular responses to a stress challenge test

For the searching of cardiovascular responses to a stress challenge associated with sleep, we used the same inclusion criteria as in the previous section.

1. We first performed four separated investigations. We searched on the following groups (see Figure 2-2):

   Group 1: "Heart rate" and "sleep" and "stress reactivity" (N= 67)

   Group 2: "Heart rate" and "sleep" and "stress recovery" (N=71)

   Group 3: "Heart rate variability" and "sleep" and "stress reactivity" (N=34)

   Group 4: "Heart rate variability" and "stress" and "recovery" (N=38)

2. Then, we joined these four groups and deleted duplicate articles ending with 122 articles, which were imported to Covidence©.

3. After screening titles and abstracts, we excluded 79 irrelevant articles.

4. After screening full text to assess for eligibility, we excluded 41 articles due to:

   a. Wrong settings (N=34),

   b. Wrong patient population (N=7)

Out of the two studies included in the qualitative review, only one study[87] involved sleep under habitual conditions. To our knowledge, this is the first article that studied the association between short sleep duration under habitual conditions and cardiovascular responses to mental stress. We included in this literature review a second study[88] that involved sleep deprivation. We decided to include it in the review because it was the only study that used orthostatic stress as a task in the stress challenge (see Table 2-2).

Sleep duration/sleep efficiency and hormonal responses to stress

For these search, we conducted two parallel searches as in previous section, one involving salivary cortisol responses to a stress challenge and the other involving salivary amylase responses associated with sleep. For cortisol, the search terms included "salivary cortisol" and "sleep" and "stress reactivity"

or "stress recovery" or "stress challenge" or "stress." For amylase, the search terms included "salivary amylase" and "sleep" and "stress reactivity" or "stress recovery" or "stress challenge" or "stress."

We then joined the two search findings and after deleting duplicates, we identified 53 potential articles to include in our qualitative review. The 53 articles were imported to Covidence©, in which we conducted the systematic review following the same steps than before:

1. First, we screened by titles and abstracts and excluded 31 irrelevant articles.
2. Then, we screened the full text to assess for eligibility and excluded 21 articles due to the following reasons:
    a. Wrong study design (N=6),
    b. Wrong settings (N=6),
    c. Non-published articles (N=3),
    d. Wrong patient population (N=4), and
    e. Sleep deprivation/restriction (N=2).

Only one article qualified to be included in the qualitative review.[89] However, we also included the two studies of sleep deprivation because of lack of studies in this topic. The sample characteristics, study design, analytical approach, and outcome measures, are shown in Table 2-3.

Results

Sleep duration/sleep efficiency and diurnal cortisol

Out of the 19 articles (Table 2-1) that were included in the qualitative review, sixteen studies (84%) were cross-sectional studies, only one study was longitudinal[90] and two were case-control studies.[91,92] Only six (32%) studies were done in North America. Sample sizes among the studies varied substantially. Four studies had a sample size >450 subjects,[80,81,93,94] the rest had fewer subjects with four having a sample size <50. Only six studies (32%) examined sleep duration as one of the main exposures (See Supplemental Table B-1), and only four of them used an objective measure of sleep duration. All studies examined sleep quality using objective measure such as PSG (5 studies) or actigraphy (4 studies) or both

(1 study), and/or subjective sleep measures using questionnaires (12 studies) such as the Karolinska Sleep Diary, the Pittsburgh Sleep Quality Index, or logbooks (See Supplemental Table B-2).

Four (21%) studies measured urinary cortisol with three of them using 24-hour urinary collection. Fifteen studies (79%) measured salivary cortisol. The number of days, number of samples per day and timing of salivary cortisol collection varied across the studies. The specimens were in general collected on weekdays, although in one study the collection was on a non-working day.[95] The number of days of collection varied between one and seven days. The number of samples collected per day also varied between one to ten samples per day. The total number of samples per collection was between 2 samples (three studies),[81,92,94] and 21 total samples (one study).[91]

Among the studies that used salivary cortisol, the derived cortisol variables used also varied. Only a few studies used features of the diurnal cortisol profile in their analysis (awakening, CAR and slope).[80,81,91,93,95-97] The others used time points of collection or only morning cortisol. The analytical approaches used for these 19 studies were also diverse. Seven studies used mixed models[80,81,90,95,96,98,99] whereas the other studies applied linear regression, ANOVA, or correlational analysis.

Sleep duration/sleep efficiency and cardiovascular responses to a stress challenge

We only found one study in this qualitative review and it is showed in Table 2-2. This study examined responses to mental stress[87] looking at the association between total sleep time and cardiovascular responses to a stress challenge in 79 undergraduate male students. Participants performed Stroop, MSIT, and speech preparation and delivery tasks, and their heart rate (HR), HR variability and blood pressure responses to the stress were evaluated. In linear regression, shorter sleep duration was associated with greater reduction in HF-HRV during and elevated diastolic blood pressure following the stress task.

Additionally, we included in this table an experimental study[88] as mentioned above. This study examined the association of sleep deprivation with cardiovascular responses in a healthy group of 16 young (range 20-28 years) and old (60-69 years) adults, all normotensives. The study found that sleep deprivation did not affect heart rate or diastolic blood pressure responses to orthostatic stress and attenuated the systolic blood pressure orthostatic response.

Sleep duration/sleep efficiency and hormonal responses to a stress challenge

We also found only one article[89] that studied sleep under habitual conditions and hormonal responses to a stress challenge. Subjects were 53 women; measures of sleep were 7-day actigraphy and subjective measures of sleep duration and quality the day before the stress test; with the outcome that was cortisol responses to a stress challenge. Participants performed a battery of 6 Stroop color-word interference tasks and cortisol was collected at 4 points: baseline, post stress, and 30 and 45 minutes after the end of the stress challenge. Objective measures of sleep quality the night before the stress challenge were associated with cortisol reactivity; participants with lower sleep quality displayed a blunted cortisol response compared to participants with higher sleep quality. This information has been extracted and is showed in Table 2-3.

Additionally, because the literature on these associations using sleep measures in the home environment is scarce, we extracted information from two experimental studies[100,101] that used sleep deprivation and added them to this table. One of these studies[100] examined the effect of sleep deprivation on salivary cortisol and amylase responses, and found higher salivary cortisol response in the sleep deprived group than in the normal sleep group. Salivary amylase response was elevated in all participants, but the groups did not differ. In the second study[101] in young healthy students, sleep restriction was associated with increased amylase at baseline but no differences in amylase response between the restricted sleep and normal sleep groups.

Discussion

The association of short sleep duration with changes in diurnal cortisol was found in only one study. The association of poor sleep quality with diurnal cortisol changes was more consistent than the association of sleep duration with diurnal cortisol. We found only two studies of hormonal and cardiovascular responses to stress challenge in subjects with habitual short sleep duration and/or poor sleep quality compared to subjects with normal sleep. This literature review shows that there is a lack of studies of the association of habitual sleep duration and/or sleep quality and the HPA axis and ANS system, and that the epidemiological studies examining these associations differ in sample size, protocol of salivary cortisol

collection and how they measured both cortisol and sleep; all of this leading to few consistencies in their results.

Diurnal salivary cortisol is a useful tool for epidemiological studies because it is feasible to obtain in large numbers of participants, the instructions are simple to follow, the collection can be done several times per day with little burden for participants, and compliance is acceptable.[102] However, using diurnal salivary cortisol as the outcome has some pitfalls that make the findings across studies difficult to compare. First, the non-linear pattern of diurnal cortisol secretion makes the protocol of salivary cortisol collection very complex. Studies with only one or two samples over the course of a day cannot produce reliable measures of diurnal cortisol. The measurement of salivary cortisol only in the morning and with less than three samples per day does not give enough information about the peak and changes of trajectory of diurnal cortisol. It has been suggested that the minimal salivary collection protocol should involve at least one sample at awakening, one at the peak of the CAR and one at bedtime.[103] Indeed, one day of salivary cortisol sampling produces results less reliable than several days of sampling. The more days on which samples are collected in a specific study the better the controlled for day-to-day variability.[104] On our literature review, almost all studies followed different protocols for salivary cortisol sampling collection differing in the number of salivary samples collected per day and in the timing of collection during the day (See Table 2-1).

In addition, studies also varied in the computation of the features of the diurnal cortisol profile (Supplemental Table B-1 and Supplemental Table B-2). Some studies averaged samples at time points others examined the deviations of cortisol during day (e.g., CAR or slope of the diurnal curve). Classically, cortisol has been analyzed using the area under the curve (AUC) for the total cortisol over the day. Most recently, studies have focused on components of the curve (CAR and slope), which have been associated with diseases. For example, higher CAR has been found in patients with depression compared to non-depressed persons.[105,106] Flattened diurnal cortisol has also been associated with mortality among patients with breast cancer,[107] and with cardiovascular disease.[41]

Few papers identified for all the searches included large sample sizes. One of the two largest studies in this review was the Whitehall II Study,[80] a cohort of 2979 London-based civil servants that used

subjective measures of sleep duration and quality and collected up to 6 cortisol samples per day. The study found an association between short sleep duration and increased salivary cortisol awakening response (CAR), and a shallower downslope in cortisol levels, as well as between sleep disturbance and a shallower downslope in cortisol levels. The second largest study of this review, the Danish study[81] of 4066 civil servants also used subjective measures of sleep quantity and quality and initially collected only 3 samples per day, the first sample 20 minutes after awakening. The study did not find an association between sleep duration and diurnal levels in cortisol, but it found inverse associations between sleep problems and morning cortisol. A subsample of this study, 387 participants, was reexamined 3 months later using same sleep parameters but with an extra salivary sample (at awakening). The study found that those with sleep problems had a flattened cortisol profile. The Whitehall II study included more men than women and a low percentage of ethnic minorities,[108] and the Danish study included mostly white people and a larger percentage of women than men.[81] As a result, the findings of these two studies may be generalizable only to similar populations.

A very few studies of sleep in habitual conditions have evaluated how habitual short sleep duration and/or poor sleep quality affects cardiovascular (heart rate, heart rate variability) and hormonal (cortisol and amylase) responses to a stress challenge test. Sleep loss may potentiate the effect of the stressors on the cardiovascular and hormonal indices. These outcomes (indices of the HPA and ANS function) are also important because people who have short sleep duration and/or poor sleep quality have to deal with different stressors when they face real life situations in everyday life.

We found only one study[87] examining the association between short sleep duration in habitual conditions and cardiovascular responses to acute stressors. This study found that short sleep duration was associated with greater reduction in HF-HRV during stress. Also few experimental studies have examined this association, in which participants underwent sleep deprivation. These studies were done in small samples of healthy young volunteers, and followed different stress challenge protocols. For example, one study showed an increase in heart rate variability during mental stress among 18 study participants who underwent acute total sleep deprivation.[67] In another controlled experimental study[68] of 20 healthy adults, the stress challenge test had a stronger effect on blood pressure reactivity but not with

heart rate in the subjects with a night of total sleep deprivation than with a night of normal sleep. In a most recent study, a randomized crossover study, 28 subjects had greater heart rate reactivity to and slower recovery from mental stress after 24 hours of total sleep deprivation than when they were not undergo sleep deprivation.[109]

In addition, we only found one study[89] that examined the association of habitual sleep quantity and sleep quality with cortisol responses to a stress challenge. This study was limited to a small sample of young-adult females and found that low sleep quality was associated with a blunted cortisol response. In experimental studies, one study[100] found sleep deprivation associated with higher cortisol reactivity to stressors and no association of sleep deprivation with salivary amylase response.

Conclusions

In total 19 epidemiological studies have examined habitual short sleep duration and/or poor sleep quality with diurnal salivary cortisol and only a couple have examined these sleep characteristics in relation to responses to a stress challenge. The current evidence of the association of habitual sleep duration and dysfunction of the HPA is not consistent especially because most of the studies have small sample sizes, the larger samples lack generalizability, and the salivary collection protocols and the cortisol indicators used in analysis vary among the studies. The association of sleep quality with diurnal salivary cortisol is more consistent than that the association of sleep duration with diurnal salivary cortisol; despite the variations in sampling methods. Practically, no studies have addressed the associations of habitual sleep duration/sleep quality and hormonal and cardiovascular responses to a stress challenge in a heterogeneous sample population. This area needs more investigation.

Data on habitual sleep in community-based samples are sparse. The few existing studies suggest some association between habitual sleep and the outcomes we have discussed, but more research is needed.

Table 2-1. Quasi-systematic literature of epidemiological studies examining associations between short sleep duration and/or poor sleep quality and diurnal cortisol

Author (Pub Year) Country	*Sample Size/ Analysis N*	*Sample Characteristics*	*Study Design/ type*	*Cortisol Sampling*	*Derived Cortisol Variables*	*Analysis/ Statistical Methods*	*Exposures/ Covariates*	*Sleep measures*	*Major Findings (significant)*
Backhaus,[91] 2004 Germany	N=29 14 with primary insomnia (DSM-IV) 15 controls	32-62 years Non-smokers BMI 19-25 Non shift workers No sleep meds No drugs No illness	Case-control	21 salivary samples (7 consecutive days): Awakening (T1), 15 min later (T2), and before bedtime (T3)	Means of the cortisol values for each of the 3 measurement points Decline	ANOVA with repeated measures	- Sleep Quality -Sleep disturbance -Sleep rumination	Pittsburgh Sleep Quality Index (PSQI) FEPS	Higher disturbance and low sleep quality and rumination associated with lower cortisol at T1. Insomniacs had lower cortisol than controls at T1 Fall of cortisol from T1 to T3 smaller for insomniacs
Eek,[93] 2012 Sweden	N=581 352 women 229 men	46 ± 11 years from different industries and one high school	Cross-sectional	3 salivary samples (one day): Awakening, 30 min later, and at 21.00 h	Mean over the day, concentration at the three points, time points, Cortisol morning peak, CAR, mean morning concentration, decline	Correlation Linear mixed models GLM univariate ANOVA	Sleep Quality	Karolinska Sleep Diary (KSD): Disturbed sleep Ease of awakening	No significant correlations
Ekstedt,[110] 2004 Sweden	N=24 12 high and 12 low burnout 14 women and 10 men	31 ± 1 years Healthy Non-smokers No medications	Cross-sectional	Plasma cortisol (8-9am) One-day salivary cortisol: awakening, 15, 30 and 60 min after awakening, 11am, 3pm, 7pm, 9pm and bedtime	Morning samples only used. Mean value of all 4 morning samples Difference sample at 0 and 60 min after awakening	linear regression	# of arousals TST Sleep efficiency	PSG: Sleep fragmentation	Higher frequency of arousals associated with higher morning salivary cortisol and plasma cortisol
Garde,[96] 2011 Denmark	N=265	Healthy worker population	Cross-sectional	3 salivary samples (one day): Awakening 30 min later, and at 20:00 h	Maximum morning concentration Evening cortisol concentration CAR Decline (slope)	Mixed models	Sleep quality: Disturbed sleep index (DSI) Awakening index	Modified KSQ Logbook Stress-Energy Inventory	Cortisol concentrations not associated with sleep

Author (Pub Year) Country	*Sample Size/ Analysis N*	*Sample Characteristics*	*Study Design/ type*	*Cortisol Sampling*	*Derived Cortisol Variables*	*Analysis/ Statistical Methods*	*Exposures/ Covariates*	*Sleep measures*	*Major Findings (significant)*
Hansen,[81] 2012 Denmark	Baseline N=4066 Follow up N=387	Population-based study Working population	Cross sectional (baseline) Longitu-dinal (follow up)	Salivary cortisol (one day) Baseline: 30 min after awakening 20:00 h Follow up: at awakening 20 min after 40 min after 20:00 h	Morning and evening cortisol CAR AUC-morning Slope	Mixed model	Poor sleep quality past 4 weeks & night before	KSQ Overall sleep quality Disturbed sleep Sleep length Awakening problems	Awakening problems and sleep quality (past 4 weeks) associated with reduced cortisol (morning and evening) *Follow-up*: Awakening problems at baseline predicted lower cortisol Disturbed sleep and awakening problems (past 4 weeks) associated with reduced CAR and flattened slope
Hanson,[98] 2000 Netherlands	N=77 36 health professionals (HP); 41 office clerks (OC)	Male and female 40 ± 5 y (HP) 33 ± 9 y (OC	Cross-sectional	2 Day-salivary cortisol. Between 8:00 h and 10.30 h 6 times (HP) 10 times (OC)	Time points	Random coefficient model	Subjective sleep quality	Groningen Sleep Quality Questionnaire	Difficulty sleeping associated with higher cortisol
Kumari,[80] 2009 UK	2751 respondents	White collar workers	Cross sectional	Salivary cortisol (A normal weekday) Awakening, 30 min, 2.5 h, 8h, and 12h after awakening and bedtime	CAR Slope	Multilevel models	Sleep duration Sleep disturbance	Log book Jenkins sleep questionnaire	Short sleep duration associated with higher CAR and shallow slope in diurnal cortisol Sleep disturbance associated with shallow slope in cortisol secretion
Lasikiewicz,[97] 2008, UK	N=147 healthy adults 68 male and 79 female	46 ± 7 years	Cross sectional	Salivary cortisol (one day) Awaking, 15, 30, 45 min after awakening, and 3, 6, 9,and 12 hours later	AUC Diurnal decline: Diurnal mean & slope Cluster 1:blunted CAR with flattened decline (78%) Cluster 2: Classical profile	MANOVA	Sleep quality	LSEQ Leeds Sleep Evaluation Questionnaire	Cluster 1 had poorer sleep quality
Luik,[94] 2015 Netherlands	N=493	Adults 57% female 56 ± 5 years	Cross-sectional	Salivary cortisol (two days) 8am	Dexamethasone suppression test .25mg PO 23.00h day 1	Successive linear regression models	24-h activity rhythm Sleep efficiency	Actigraphy Log book	Lower stability of the 24h activity rhythm is associated with enhanced negative feedback of cortisol

Author (Pub Year) Country	*Sample Size/ Analysis N*	*Sample Characteristics*	*Study Design/ type*	*Cortisol Sampling*	*Derived Cortisol Variables*	*Analysis/ Statistical Methods*	*Exposures/ Covariates*	*Sleep measures*	*Major Findings (significant)*
Prinz,[111] 2000 USA	N=98 60 women 28 men	55-82 years non obese healthy community-living	Cross-sectional	24-hour urine	Urinary free cortisol (UFC) Plasma cortisol	Multiple regressions	EEG beta activity Sleep efficiency	PSG	UFC levels were not associated with sleep in baseline conditions
Prinz,[112] 2001 USA	N=42 healthy women	Menopause 50% with HRT no apnea normal range of depression and anxiety scores	Cross-sectional	24-hour urine	Urinary free cortisol (UFC)	Multiple regressions	Sleep quality Sleep efficiency REM sleep	Pittsburgh Sleep Quality Index (PSQI) PSG	Higher UFC levels were consistently associated with lowered sleep efficiency and less REM sleep in non HRT women under baseline conditions
Rao,[79] 2013 USA	N=325 men	Mean age: 76.6 ± 5.5 years The MrOS Sleep Study	Cross-sectional	24-hour urine	Urinary free cortisol (UFC)	Multiple linear regression	Sleep Duration Sleep Efficiency	Sleep duration, measured objectively (by actigraphy) and subjectively (by self-report) Slow wave sleep (by PSG)	No association of urinary cortisol with objective sleep duration/quality Inverse association of urinary cortisol with subjective sleep duration
Rueggeberg,[90] 2012 Canada	N=157	Older adults Community dwelling	Longitudinal Baseline, 2 years after, and 4 years after	3-day salivary cortisol: Awakening 30 min after 2 pm, 4 pm and before bedtime	AUC (total diurnal cortisol)	Cross-lagged panel analyses with regression models Growth-curve analysis	Sleep duration Sleep quality	Brief Pittsburgh Sleep Quality index	Cortisol secretion increased over 4 years of study. Shorter sleep at baseline and 2-year follow-up associated with higher cortisol over the subsequent 2 years
Rydstedt,[113] 2013 UK	N=76 Men and women	Mean age= 45.8 years white collar workers	Cross-sectional	14 salivary cortisol samples: 7 days collection: Awakening and 22:00 hr	Time points	Factorial ANOVA	Sleep quality Job strain	Pittsburgh Sleep Quality index (PSQI)	Unadjusted model: lower sleep quality associated with reduced morning cortisol Adjusted (age and sex) model: no association
Seelig,[92] 2013 Switzerland,	N=13 (insomnia by DSM-IV) N=12 (matched control)	Healthy normal weight women	Case-control	Salivary cortisol (two samples) 11.30pm and 6am	Time points (Midnight and early morning)	Correlation analyses	Time of sleep Sleep efficiency Number of arousals Sleep stages	2 night home PSG	Sleep architecture of cases and controls did not differ. Midnight salivary cortisol levels were higher in insomniacs than in non-insomniacs

Author (Pub Year) Country	*Sample Size/ Analysis N*	*Sample Characteristics*	*Study Design/ type*	*Cortisol Sampling*	*Derived Cortisol Variables*	*Analysis/ Statistical Methods*	*Exposures/ Covariates*	*Sleep measures*	*Major Findings (significant)*
Shaver,[114] 2002 USA	N=53 (mid-40s, white and college educated women)	Insomnia of at least 3 months (N=39) vs. non-insomnia (N=14) Community dwelling *Exclusions:* any other sleep disorder	Cross-sectional	3 non-consecutive days: 3-hour urinary cortisol before their usual bedtime and first-voided urine specimen each morning	Urinary cortisol AM, PM and AM-PM difference	Correlation analyses	Insomnia PSG patterns NREM and REM sleep Sleep efficiency	6-night Home sleep PSG	Morning-to-evening difference scores for cortisol were greater in insomniac group than in control group
Vargas,[99] 2014 USA	N=58	Healthy young adults Age: 18 ± 1	Cross-sectional	8 salivary samples (2 consecutive weekdays) At awakening 30, 45 and 60 min after awakening	AUC Awakening (intercept) Cortisol reactivity (slope) from awakening =CAR	Mixed models	Total sleep time (TST) Sleep onset latency Wake after sleep onset (WASO) Wake time Sleep Quality	The Pittsburgh Sleep Quality index (PSQI) Core Consensus Sleep Diary (Core CSD)	Lower TST was associated with lower cortisol at awakening and with greater CAR
Zhang,[115] 2011 China	N=101 Middle-age adults	Exclusion: sleep disorders Psychiatric & medical conditions	Cross-sectional	3 day one-morning salivary cortisol	Average morning awakening cortisol	Linear regression Adjustment	Sleep duration Sleep efficiency	Actigraphy	No association
Zhang,[95] 2014 China	N=244 middle age adults	175 insomniacs (DSM-IV) 69 non-insomniacs 46 ± 4 years Community base	Cross-sectional	Salivary cortisol samples on a one free day Awakening, 30, 60 and 90 min after awakening Noon, 4pm, 10pm	AUC of CAR AUCg (ground) AUCi (increase) Diurnal cortisol (slope) Time points	GEE models Mixed models	Sleep efficiency	3-day Actigraphy Sleep Dairy	Insomnia was associated with increased CAR activity Poor subjective sleep quality was associated with higher evening cortisol levels in the insomnia group

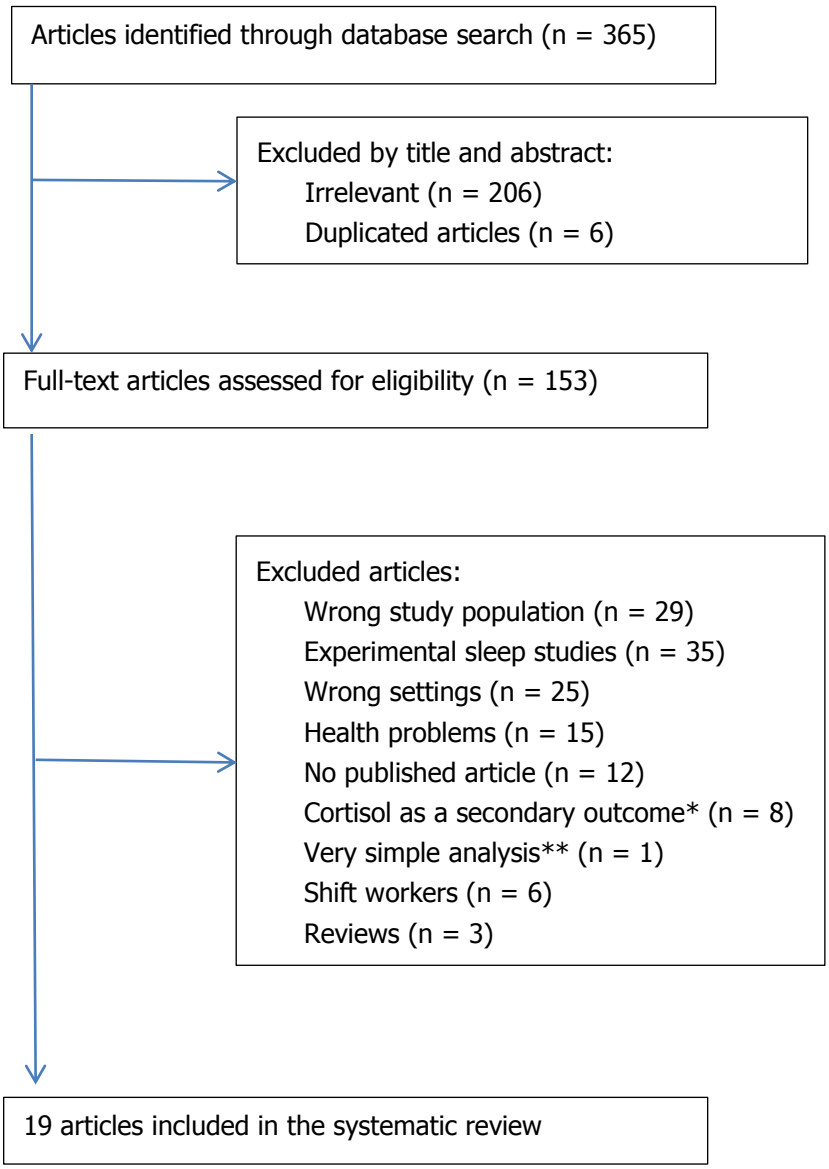
Table 2-2. Quasi-systematic literature of studies examining associations between short sleep duration and/or poor sleep quality and cardiovascular responses to a stress challenge

Author (Pub Year/County)	Sample Size/ Analysis N	Sample Characteristics	Study Design/Type	Analysis/ Statistical Methods	Stress Challenge Task	Sleep measure	Outcome Measure(s)	Major Findings
Mezick,[87] 2013 USA	N= 79 Age=18-30 years	Undergraduate men Short sleepers (47%)	Cross-sectional	Standardized residual (change from baseline to task controlling for baseline) Linear regression	Stroop MSIT Speech preparation and delivery	Total sleep time (actigraphy) Task respiration rate	HF-HRV HR Blood pressure Reactivity recovery	Shorter TST was associated with greater reduction in HF-HRV during and elevated diastolic BP following stress task
Robillard,[88] 2011 Canada	N=16 8 young (20-28) 8 elderly (60-69)	exclusion: sleep apnea low sleep efficiency	Cross-over counterbalance	Mixed factorial ANOVA with two repeated measures	Orthostic stress	Night sleep and night sleep deprivation	HR SBP DBP	SD raises SBP and DBP in the older adults SD did not affect HR and DBP responses to position change SD reversed the SBP orthostatic response (attenuated)in both young and older adults

Table 2-3. Quasi-systematic literature of studies examining associations between short sleep duration and/or poor sleep quality and hormonal responses to a stress challenge test

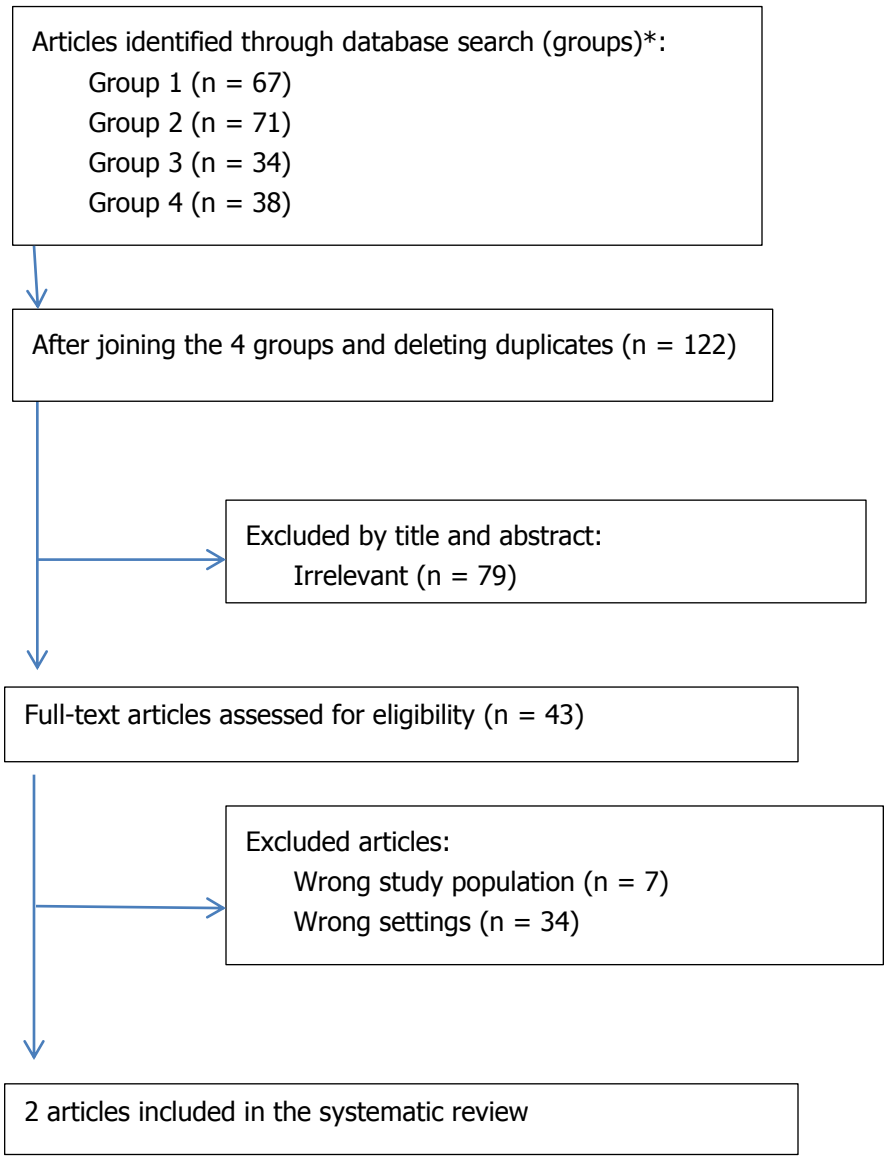
Author (Pub Year/Country)	***Sample Size/ Analysis N***	***Sample Characteristics***	***Study Design/ type***	***Analysis/ Statistical Methods***	***Stress Challenge (SC) Task***	***Sleep measures***	***Outcome Measure (s)***	***Major Findings***
Wright,[89] 2007 USA	N=53 Women	Age: 37 ± 10 Exclusion: CVD, cancer, depression, use of medications other than birth control	Cross-sectional	Multiple linear regression	A battery of 6 Stroop color-word interference tasks	Sleep Duration Sleep Quality the night before and a previous week. Objective: 7-day actigraphy Subjective: Pittsburgh sleep diary	Cortisol reactivity adjusted for baseline Four samples: baseline, post task and 30 and 45 min after	Sleep the night before: Sleep quality (obj) was associated with cortisol reactivity Sleep quantity (obj) was not Participants with higher quality sleep experienced cortisol reactivity whereas participants with lower quality sleep experienced a blunted cortisol response No association with subjective measures Sleep over the week prior to the SC had no effect
Minkel,[100] 2014 USA	N=26 14 men and 12 women	Range age 22-49 years; Community sample Healthy participants	Randomized Intervention Total sleep deprivation (12) control: 9 hours sleep (14)	Mixed model ANOVA with random effect	Trier Social Stress Test (TSST)	Sleep deprivation	Salivary cortisol and salivary amylase 20 and 5 min before and 5,20,40 min after stressor	Higher salivary cortisol following a social stressor in sleep-deprived than in normal sleep participants. Elevated alpha amylase activity overall in response to the TSST but no evidence of between-groups difference
O'Leary,[101] 2015 USA	108 (80 female) 17-22 years normal BMI	Healthy students	Randomized Sleep restriction vs. resting sleep	Mixed factorial design	Mental arithmetic task	Sleep restriction	Salivary amylase samples: pre and post stress	Sleep restriction was associated with increased basal amylase No difference in amylase response between the restricted sleep and normal sleep groups

Figure 2-1. Flow chart of articles selected for sleep duration/sleep efficiency and diurnal cortisol



* The cortisol outcomes were described but not analyzed in the papers.
** The analysis involved a simple t-test and no adjustment for covariates.

Figure 2-2. Flow Chart of articles selected for sleep duration/sleep efficiency and cardiovascular responses to a stress challenge



*Group 1: "Heart rate" and "sleep" and "stress reactivity;" Group 2: "Heart rate" and "sleep" and "stress recovery;" Group 3: "Heart rate variability" and "sleep" and "stress reactivity;" and Group 4: "Heart rate variability" and "stress" and "recovery"

3. CHAPTER 3. ASSSOCIATIONS OF SLEEP DURATION AND QUALITY WITH ACTIVATION OF THE HYPOTHALAMIC-PITUITARY ADRENOCORTICAL (HPA) AXIS

Abstract

Context: Short sleep duration and poor sleep quality are associated with cardiovascular outcomes. One mechanism proposed to explain this association is altered diurnal cortisol secretion.

Objective: To examine the associations of sleep duration and sleep efficiency with diurnal salivary cortisol levels, taking insomnia and other factors into account.

Design: Cross-sectional analysis using data from the Multi-Ethnic Study of Atherosclerosis (MESA), a population-based study. In 2010-2012, actigraphy-based measures of sleep duration and efficiency and up to 16 diurnal salivary cortisol samples over 2 days were collected in white, African-American, and Hispanic study participants aged 54-93 years old (n = 600 with analyzable data). Piecewise mixed linear models were used to assess the associations of sleep parameters with components of the daily cortisol profile. We adjusted the models for time of wake-up, day of salivary collection, age, gender, race/ethnicity, income-wealth index, behavioral factors, chronic health problems, and other sleep characteristics.

Results: Shorter average sleep duration (<6 hours/night) was associated with less pronounced late decline in cortisol (2.2% difference in slope; 95% CI 0.8 to 3.7; P≤0.01) and a less pronounced wake-to-bed slope (2.2% difference; 95% CI 1.0, 3.4; P ≤0.001) compared to longer sleep duration (≥6 hours/night). Lower sleep efficiency (<85%) was associated with less pronounced early decline in cortisol (29.0% difference in slope; 95% CI 4.1, 59.7; P <0.05) compared to higher sleep efficiency (≥85%). Study participants reporting insomnia (n=209) had a flatter cortisol awakening response (CAR) (−16.1% difference in slope; 95% CI −34.6 to −0.1; P <0.05) than those without insomnia.

Conclusions: Shorter sleep duration, lower sleep efficiency, and insomnia are associated with alterations in diurnal cortisol levels consistent with changes in hypothalamic-pituitary-adrenal regulation.

Introduction

Cardiovascular disease (CVD) has been one of the major health outcomes linked to short sleep duration. Women with self-reported sleep duration of ≤ 5 hours per night had higher risk of CHD than women who slept 8 hours per night. [8,18] Short sleep duration has also been associated with CVD risk factors including hypertension,[13] diabetes,[116,117] and obesity.[12,118] Subclinical CVD as indexed by coronary artery calcium (CAC) and carotid intima-media thickness (CIMT) has also been linked to short sleep duration. In the CARDIA study, each additional hour of sleep was associated with lower risk of CAC.[19] Short sleep duration was also associated with greater CIMT,[20] but only among men. In the Whitehall Study, participants with shorter sleep duration had higher rate of CVD mortality (HR 2.04, 95% CI 1.20−3.49)[119] compared to participants with longer sleep duration.

In addition to sleep duration, poor sleep quality either assessed through objective measurements of low sleep efficiency on actigraphy or subjectively through reports in insomnia has also been associated with adverse CVD outcomes. Poor sleep quality as assessed by decreased slow wave sleep (SWS) was associated with hypertension[28] and obesity[29] among adults. Poor sleep quality as assessed by low sleep efficiency and measured by actigraphy was also associated with higher blood pressure among healthy adolescents.[30] Insomnia symptoms were associated with a higher rate of acute myocardial infarction in one large cohort study of men and women.[26] Insomnia symptoms were also associated with higher risk of CVD[120] and CHD mortality,[121] only in men.

One potential mechanistic link between sleep and CVD risk involves alteration of the function of the hypothalamic-pituitary-adrenal (HPA) axis with consequences for cortisol levels over the day. The secretion of cortisol, end product of the HPA axis, during the day exhibits a marked circadian pattern with a sharp increase within the first 30 minutes after awakening termed as the cortisol awakening response (CAR).[53] The CAR is followed by a steep decline within the 2 hours after awakening (early decline) and then a slow decline over the rest of the day (late decline).[52,54,122] Studies of the association between sleep characteristics and measures of HPA axis functioning have been inconsistent. In small experimental studies in young volunteers, both total and partial sleep deprivation were followed by increases in plasma or salivary evening cortisol.[47-49] However, plasma cortisol level was lower the day following a night of

sleep deprivation in a study of 10 young healthy men,[50] and the cortisol awakening response (CAR) was not affected in a study of imposed sleep disturbance in 13 young women.[51] Observational studies investigating the associations between sleep duration and/or sleep quality (sleep efficiency or insomnia symptoms) and diurnal cortisol level have also been inconclusive. At least two epidemiological studies with large sample size found associations between sleep duration and/or sleep quality and cortisol. For example, in the Whitehall study, short sleep duration was associated with a more pronounced CAR and a less pronounced decline in cortisol,[80] and poor sleep quality was associated with a less pronounced decline in cortisol.[80,81] Other studies found no association between cortisol and sleep.[79,93,115]

Prolonged sleep deprivation, which may result from intense psychological stress, has been thought to predict an allostatic overload involving the hypothalamic-pituitary-adrenal axis (HPA),and reducing parasympathetic tone, and increasing inflammatory markers.[34] The HPA axis, one of the major neuroendocrine systems in response to stress, has also been linked to some health outcomes. For example, whereas subjects with higher levels of cognitive function keep healthy diurnal cortisol patterns,[123] studies have found higher cortisol awakening response (CAR) in patients with current, remittent and at-risk depression compared to normal persons,[105,106] and also blunted CAR in patients with depression compared to normal subjects.[124] Flattened diurnal cortisol has also been associated with depression in patients with CHD[125] and with cardiovascular and non-cardiovascular mortality.[41,107] Dysregulation of the HPA has also been linked to diabetes,[126,127] and atherosclerosis.[42]

It has also been suggested that short sleep duration is associated with adverse cardiometabolic outcomes when it is accompanied by poor sleep quality and insomnia symptoms.[128] In support of this concept, the joint presence of insomnia and short sleep duration has been linked to higher risk of type-2 diabetes,[31] hypertension, [33] and mortality.[129] The relationship between insomnia and cortisol has been investigated in a few small studies and findings were inconsistent. Whereas in two small case-control studies,[130,131] insomnia was associated with higher evening cortisol level, in other studies insomnia was associated with lower awakening cortisol,[91] or no association was found.[132]

The inconsistencies in the association of sleep duration and sleep quality with diurnal cortisol may reflect differences in the samples, small sample sizes, measurement differences, or differences in

conceptualizing sleep exposures. Prior epidemiological studies have been limited by lack of objective measures of sleep duration and quality, lack of cortisol measures over the whole day, and lack of a comprehensive set of potential confounders.

We hypothesized that participants with short sleep duration or poor sleep quality would have a less pronounced cortisol awakening response (CAR) and a shallower cortisol decline during the day than those with moderate duration of sleep. We further hypothesized that insomnia is a moderator in the association between sleep duration and cortisol, such that short sleep duration is associated with cortisol alterations only when insomnia is present. We conducted our study in a large population-based sample using objective measures of sleep duration and quality and multiple measures of salivary cortisol collected over two days.

Methods

Participants

The Multi-Ethnic Study of Atherosclerosis (MESA) is a longitudinal study designed to investigate risk factors for subclinical CVD and its progression to clinical CVD among middle-aged and older adults recruited at six field centers in the United States. In 2000-2002, MESA recruited 6814 men and women 44-84 years old and free of clinically diagnosed CVD.[133] At each of 5 exams, participants provided information on medical history, behavioral habits, and psychosocial factors, and anthropometric measures, blood pressure readings, and assessments of cardiovascular risk status were obtained. Cohort members were also invited to participate in ancillary studies; two of them were the MESA Stress Study and the Sleep Study that were concomitant with the MESA Exam 5 (2010-2012). The MESA Study was approved by the IRB at each of the field centers, and all participants gave written informed consent at each exam.

The MESA Stress ancillary study enrolled 1,082 MESA participants at the New York, Los Angeles, and Baltimore field centers during Exam 5. MESA Stress involved two-day collection of salivary cortisol samples and a stress challenge test in a different day. At the three MESA Stress field centers, 1083 subjects participated in the Sleep Study; of these 622 also participated in the Stress Study. Of the 622

participants who completed both the Stress and Sleep protocols, we excluded those participants with extreme values for sleep hours (< 3 hours or ≥ 9 hours). Our final sample comprised 600 participants.

Collection of salivary cortisol samples

Participants were instructed to collect eight salivary samples per day over 2 weekdays, resulting in a maximum of 16 samples per person. They were instructed to chew a cotton swab gently for 1-2 minutes and then place it in a numerated salivette previously provided. The first sample was taken upon awakening, the second sample 30 min later, the third sample one hour after breakfast, the fourth sample around 10 am, the fifth sample at noon, the sixth sample around 4 pm, the seventh sample around 6 pm (or before dinner if dinner occurred before 6 pm), and the eighth sample right before bed. Participants were provided with a digital clock to record the time of salivary sample collection, and they were asked to write down the times of sample collection for each sample on a daily questionnaire.

Measurement of salivary cortisol

Salivary samples were stored at −20°C until analysis. Before biochemical analysis, samples were thawed and centrifuged at 3000 rpm for three minutes to obtain clear saliva with low viscosity. Salivary cortisol level was determined using a commercially available chemiluminescence assay with high sensitivity (0.16 ng/mL) (IBL Hamburg, Germany). Intra- and inter-assay coefficients of variation for the essay are <8%. Cortisol was measured in nmol/L (nanomoles per liter).

The MESA Sleep Study was the second ancillary study also conducted at Exam 5. All MESA participants except those reporting regular use of oral airways support devices, nocturnal oxygen or nightly continuous positive airway pressure were eligible to participate. The protocol included 7-day actigraphy (Actiwatch Spectrum, Philips Respironics) together with 1-night home polysomnography (PSG), a sleep diary, and a sleep questionnaire. Actigraphy is an objective method of assessing sleep-wake parameters in natural settings. It contains a chip that senses the signals generated by movements of the wrist and translates them into "activity" counts, which are translated into voltage that are gathered continuously and stored into epochs of one minute. While wearing the actigraph, participants recorded on the sleep diary information on bed and wake times, sleep onset, naps, actigraphy removal, and other unusual events. Actigraphic data during 30-second epochs were scored as sleep or wake by Actiware-

Sleep version 5.59 analysis software (Mini Mitter Co., Inc.). Summary data for average weekly sleep duration and sleep efficiency were generated using a validated algorithm[134]. Intrascorer intraclass correlation coefficients for average sleep duration and sleep efficiency were 0.91 and 0.97.

Outcome Variables

The diurnal cortisol profile was determined by using up to 16 measures of cortisol per participant from the two-day salivary cortisol collection. Samples were collected between awake and 16 hours after awakening. Samples taken after 16 hours of awakening were excluded because cortisol starts to rise again after 16 hours of awakening. We used the self-reported first sample collection time as an approximation of the wakeup time in order to keep comparability with other analyses done with the same dataset. We assigned time equal 0 hours to the first sample as time since wakeup. The distribution of the difference between the wakeup time and the first sample time of the day is shown in Supplemental Table C-1. The derived cortisol variables were wakeup cortisol (cortisol awakening level), cortisol awakening response (CAR) and early decline slope and late decline slope (See Figure 3-1)**.** Additionally, we used two summary measures of cortisol: area under the curve (AUC) for a 16-hour day, and the overall decline slope (wake-to-bed slope).

Exposure Variables

Objective measures of sleep duration and sleep quality were averaged across at least four weekdays and one weekend day of actigraphy. Sleep duration was defined as the average time the participant subject spent asleep between sleep onset (sleep start time) and morning wakening (sleep end time) while in bed after "lights off." It was calculated by taking the sum of all time asleep across the recording and dividing by the total number of main sleep periods. Sleep duration was estimated as a continuous variable (range 3 to <9 hours); in addition less than 6 hours was defined as "short sleep." Other cut points for short sleep duration (5 and 7 hours) were also analyzed in sensitivity analyses. Sleep efficiency, an objective measure of sleep quality, was defined as the percentage of time in bed after "lights off" spent sleeping. It was calculated by taking the sum of all sleep time divided by the sum of all in bedtime during main sleep intervals across the recording and multiplied by 100 to obtain a percentage. Sleep efficiency was analyzed as a continuous variable; in addition less than 85% was defined as low sleep efficiency.

Insomnia, a subjective measure of sleep quality, was assessed based on self-report using the Women's Health Initiative Insomnia Rating Scale (WHIIRS), a 5-item questionnaire: 1) "Having trouble falling asleep," 2) "Waking up several times a night," 3) "Waking up earlier than planned," 4) "Having trouble getting back to sleep after waking up too early," and 5) "How was your typical night's sleep." Participants were asked to rate how frequently they experienced those difficulties over the past four weeks. Each question was scored on a 5-point scale (from 0−4). The summary score ranged from 0 to 20. A score of ≥ 9 is considered clinically significant insomnia. [135]

Covariates

Age, sex, race/ethnicity, awakening time, income-wealth index were examined. Family income and wealth were assessed by self-report at Exam 5. A 9-point income-wealth index was created by summing responses from a 5-category family income measure and a 5-point family wealth measure.[136] Smoking was assessed at Exam 5 by using the following categories: "never smoked"; "former smoker quit more than 1 year ago"; "former smoker quit less than 1 year ago"; "current smoker"; and "do not know." These responses were categorized as never smoker; former smoker quit more than 1 year ago; and current smoker and former smoker quit less than 1 year ago, as one category, because the category of current smokers included a few participants. Alcohol consumption was assessed using the response to the following: "Do you presently drink alcoholic beverages?:" Yes/No. Body mass index (BMI) (kg/m^2) was calculated as weight in kilograms divided by height in meters squared.

Cynical hostility was measured at Exam 5 using an 8-item subscale of the full Cook-Medley Hostility Scale[137] with higher scores indicating higher levels of cynical-hostility. Cynical-hostility was analyzed as tertiles. Depression was assessed at Exam 5 using the 20-item Center for Epidemiology Studies-Depression (CES-D) scale[138] with higher scores indicating higher levels of depressive symptoms. A CES-D score of ≥16 was defined as depression, consistent with mild-to-moderate depression in the population.[139] Depression scores were entered in the models as a continuous variable.

Other covariates included prevalent diabetes and hypertension as of Exam 5, and use of certain medications. We grouped medications used for sleep and/or mood (non−tricyclic antidepressants, tricyclic anti-depressants, norepinephrine-dopamine reuptake inhibitors, serotonin antagonists and

reuptake inhibitors, serotonin-norepinephrine reuptake inhibitors, selective serotonin reuptake inhibitors, anti-psychotic medications, and benzodiazepines); oral and inhaled steroids; and hormone replacement therapy (HRT) (estrogens, including vaginal creams; progestins; and premarin (conjugated estrogens)).

The apnea/hypopnea index (AHI) was calculated based on data from the overnight PSG. Apnea was defined as complete or near complete cessation of airflow for ≥10 seconds, and hypopneas as at least 30% below baseline breathing amplitude for ≥10 seconds. We included only apneas and hypopneas that were associated with ≥3% desaturation. The AHI was calculated as the total number of apneas and hypopneas per hour of sleep. It was included in the models as a continuous variable.

Analysis

A total of 1082 participants were enrolled in the MESA Stress Study 2, providing 2164 days of salivary cortisol data and 17312 samples. We excluded observations or participants as follows: First, we excluded 6 days of data (48 samples) missing sampling times (since wakeup) for the entire day of data collection; second, we excluded days that were missing cortisol value for the entire day of data collection (24 days and 192 samples); third, we excluded individual samples with missing sampling times (since wakeup) or missing cortisol values (1040 samples). Fourth, we excluded samples with cortisol values over 100 nmol/L (113 samples) and samples with cortisol values of 0 nmol/L (4 samples). Fifth, we excluded participants with no measure of sleep duration because they did not participate in the Sleep Study (447 participants). Finally, we excluded participants who had slept ≤3 hours (11 participants) or >9 hours (11 participants) because these two groups had few participants < 2% in each group. Our final analytical sample included 600 participants over 1198 days with 8985 samples. (Supplemental Figure C-1 illustrates these exclusions). In our final dataset, we found participants with abnormal wakeup times; 4 subjects (6 days) had a very early wakeup (before 3:00 am) and other 4 subjects (4 days) a very late wakeup (after 11:00 am). We did not exclude these participants, but we did a sensitivity analysis and found no differences in including or not this group of participants. Most participants (88%) in our dataset collected at least 14 salivary samples over two days (see

Supplemental Table C-2). Most participants collected at least 7 salivary samples per day (day 1: 92% and day 2: 89%) (Supplemental Table C-3).

Associations of sleep measures with features of the daily cortisol curve before and after adjustment for covariates were examined using piecewise linear regression mixed models consistent with Ranjit (2009).[140] We used a piecewise linear mixed model with two knots at 0.5 hours and at 2 hours after wake-up to capture the inflections of the diurnal cortisol profile Figure 3-1. The rational to select the two knots at 0.5 after wakeup as the first knot and 2 hours after wakeup as the second knot is because salivary cortisol samples were collected at 30 minutes and two hours after wakeup. Most participants had peak cortisol level at 0.5 hours (2^{nd} sample) and then there is a steep decline between the 2^{nd} and 3^{rd} samples with the declining rate gradually leveling over the 3^{rd} through the 8^{th} samples (See Table 3-1 and Figure 3-2). The distribution of the salivary sample collection time (hours) of our data set is shown in *For instance, on day 1, only 2 participants provided one sample for the whole day whereas 399 participants provided 8 samples for the whole day.

Supplemental Table C-4. The two knots produce a three-spline model. We computed the cortisol features of this sample by pooling all the data across all the day for the individual (up to 16 samples per participant). Using all cortisol samples in the model allows us to investigate associations of each exposure with the diurnal cortisol profile as a whole including the main features: wakeup, CAR, early decline and late decline. This approach as opposed to modeling each of the features separately and/or averaging the features computed for each day has more statistical efficiency. The cortisol features associated with the exposure are derived from the parameter estimates from the fixed effect in the model. We included the random slopes for only the first and third pieces of spline because when we included the second spline the models did not converge. The objective of our analysis was to identify differences by exposure in the cortisol curve. Cortisol values were log transformed (log (nmol/L)) to approximate it to a normal distribution, therefore, the exponentiated coefficients from the models were construed as percent differences. Positive percent differences in the wake-up cortisol indicate higher cortisol levels; positive percent differences in the CAR indicate a sharper rise in cortisol; and positive percent differences in the early and late decline indicate a flatter decline in cortisol.

We computed several cortisol features. The cortisol awakening response (CAR) was calculated as the difference between the wake up cortisol levels and the levels at 30 minutes post-awakening. The early decline slope (between 30 minutes and 2 hours post-awakening) and the late decline slope (between 2 hours post-awakening and bedtime) were calculated as the average hourly rate of decline for the given time period. The overall slope was calculated as the average hourly rate of decline from wakeup to bedtime (excluding the 2nd sample when calculated). AUC was defined as the area under the cortisol daily curve standardized by 16 hours.

We examined the shape of the cortisol profile over the course of the day using locally estimated scatter plot smoothing (LOESS)[141] following previous studies,[136] for the whole population and by gender (Figure 3-2), and also stratified by sleep duration (Supplemental Figure C-2) and sleep quality (Supplemental Figure C-3) as well as by race/ethnicity and age (Supplemental Figure C-4).

We examined participant characteristics and cortisol measures by categories of sleep duration and sleep efficiency. The statistical significance of differences in cortisol variables and covariates by sleep duration and sleep efficiency category was evaluated by ANOVA for continuous variables or Kruskall Wallis when variables had a skewed distribution and chi-square tests for categorical variables.

All covariates were centered with respect to the grand mean when included in the models. Centering supports interpretation of the model intercept for an average population.[142] All models were adjusted for time of wake-up, first vs. second day of salivary collection, age, gender, race/ethnicity, and income-wealth index.

Analyses of sleep duration and sleep quality were performed using both categorical and continuous measures. In the analysis of sleep duration, model 1 was adjusted for first vs. second day of salivary collection, wake-up time, gender, age, race/ethnicity, and income-wealth index. Model 2 was adjusted for covariates included in model 1 plus BMI, smoking, alcohol consumption, medications, and AHI. Model 3 included covariates in model 2 plus hypertension, diabetes, and depression. Model 4 (fully adjusted model) for sleep duration included covariates in model 3 plus sleep efficiency and for sleep efficiency as the predictor model 4 included sleep duration as a covariate. All covariates included in the models were entered as main effect and as interacting with pieces of the daily curve.

We also calculated the wake-to-bed slope in cortisol level to analyze the associations of sleep duration and sleep quality with a summary measure of cortisol decline over the day. This summary measure, reported in prior studies,[143] is the slope of the decline of cortisol between wake-up and bedtime. Consistent with previous study, we estimated the wake-to-bed slope within the mixed model (excluding the 30 minute sample), and used the same covariates in the models as described above. In addition, we investigated the cortisol area under the curve (AUC) for a 16- hour day, a second summary measure of the daily cortisol profile, and estimated it within the mixed model.

We explored effect modification using stratified analyses to study the role of insomnia in the association of sleep duration with alterations in the diurnal cortisol level. A test for interaction between insomnia symptoms and sleep duration was used to test whether the association of sleep duration with cortisol differed between subjects with and without insomnia. We first examined whether the insomnia

covariate was statistically significant with P <.2, and then we included in the model the main effect, two-way interactions with the three spline pieces, and three-way interactions (insomnia, sleep duration, each of the three spline pieces).

Test of interaction between gender, age (54 to <68, 68 to < 75 and 75 years old or more) and race/ethnicity (whites, African Americans and Hispanics) and sleep duration were also examined including three way interaction terms for the slopes.

We performed secondary analyses in which we restricted our sample to participants younger than 75 years, to those without depression, to nonusers of oral/inhaled steroids, to nonusers of antidepressants, and to participants with an apnea hypopnea index of less than 15 (without moderate or severe apnea). We also investigated alternative cut points of both sleep duration and sleep efficiency.

Results

Our overall sample consisted of 316 (53%) women and 284 (47%) men from three different race/ethnic backgrounds, Hispanic (40%), African-American (32%), and white (28%), and the mean age was 69.2 (S.D. 9) years. Table 3-1 shows summary characteristics for the 600 participants included in the analyses by sleep duration and sleep efficiency. Participants were categorized as "short" sleepers (<6 hours) (n=202) and "longer" sleepers (≥6 to <9 hours) (n=398), and as "low" sleep efficiency (<85%) (n=60) and "higher" sleep efficiency (≥85%) (n=540). The median (interquartiles: Q1−Q3) evening cortisol (at bedtime) was higher among short sleepers (3.6 nmol/L, 2.1−7.0) compared to longer sleepers (3.3 nmol/L, 1.9−5.3; P=.03). The normal median for the evening cortisol in this assay is approximately 2.0 nmol/L (10^{th} percentile=0.1− 90^{th} percentile=6.0). Characteristics of the study participants by insomnia symptoms are also shown in Table 3-2. Supplemental Table C-5 shows selected characteristics of participant demographics by cortisol data collection.

Sleep Duration

Characteristics of participants by mean sleep duration (hours) are shown in Supplemental Table C-6. Men, African-Americans, low income participants, and participants who were obese, diabetic, used inhaled steroids, or had low sleep efficiency had lower average sleep duration than their counterparts.

Table 3-3, first column, shows percent differences and 95% confidence intervals (CI) between short

and longer sleep duration in features of the diurnal cortisol profile, and the wake-to-bed slope and AUC. Short sleep duration was associated with less pronounced late decline in all models (model 4, 2.2% difference in slope; 95% CI 0.8−3.7; P ≤.01) and with less pronounced wake-to-bed slope in all models (model 4, 2.2% difference; 95% CI 1.0−3.4; P ≤.001). Short sleep duration was not associated with AUC. Consistent patterns were observed when sleep duration was investigated as a continuous variable (Supplemental Table C-7, first column).

Sleep efficiency

Sleep efficiency ranged from 71.62% to 96.98%. Table 3-1 shows that participants with low sleep efficiency had lower levels of cortisol at awakening (p-value =0.02) and 30 minutes after (p-value =0.01) compared to those with higher sleep efficiency, but there was no difference in the evening cortisol between the two groups.

Low sleep efficiency was associated with less pronounced early decline in all models (model 4, 29.0% difference in slope; 95% CI 4.1−59.7; P ≤.05) (Table 3-3, second column). Sleep efficiency was not associated with the wake-to-bed slope or AUC. Consistent patterns were observed when sleep efficiency was investigated as a continuous variable (Supplemental Table C-7, second column).

Insomnia

Table 3-2 shows summary characteristics for the 591 participants who had insomnia data. In this analytic sample, 209 participants (35%) reported insomnia symptoms. This group was more likely to include women, to be in the lowest categories of the income-wealth index, and to report higher scores of depression symptoms and hostility compared to those without insomnia symptoms.

Table 3-4 shows the associations between insomnia and cortisol levels in the full sample and stratified by short vs. longer sleep duration. Overall, insomnia was associated with less pronounced CAR in models 1, 2, and 4 (model 4, −16.1% difference; 95% CI −34.6 to −0.1; P <.05) compared to those without insomnia (Table 3-4, first column). In the subsample of short sleepers, insomnia was associated with less pronounced CAR compared to those without insomnia in all models (model 4, −37.7% difference; 95% CI −79.4 to −5.7; P <.05) (Table 3-4, second column). In the subsample of longer sleepers, insomnia was associated with a more pronounced late decline in cortisol and more pronounced

wake-to-bed slope compared to those without insomnia in all models (Table 3-4, third column).

We also compared the association between sleep duration and salivary cortisol in participants with and without insomnia. In the group with insomnia (Table 3-5, first column), short sleep duration was associated with less pronounced CAR in models 3 and 4 and with less pronounced late decline in cortisol in all models (model 4, 3.6% difference in slope; 95% CI 1.2−6.0; P ≤.01) compared to longer sleep duration. This was not observed in the group without insomnia (Table 3-5, second column). Both the groups with and without insomnia showed less steepness in the wake-to-bed cortisol slope in those with short vs. longer sleep duration (Table 3-5). The statistical test for interaction between insomnia and sleep duration was of marginal significance (P for interaction = 0.1).

Secondary analyses

Participants with depression represented 17% (N=600) of our sample. Only the second sample of salivary cortisol (taken 30 minutes after awake) was different among those who had CES-D ≥16 (median=18.9 nmol/L, Q1−Q3=12.8−28.9) compared to those with CES-D <16 (median=22.3 nmol/L, Q1−Q3=15.5−31.5). Salivary cortisol levels did not differ between participants with depression taking antidepressants compared to those who were not, or between those with hypertension or diabetes compared to those without hypertension or diabetes.

Results of analyses restricted to participants younger than 75 years old, those without depression, non-users of antidepressants, non-users of oral/inhaled steroids, and participants without moderate sleep apnea were not materially different from the unrestricted analyses. Our results did not differ from the unrestricted sample when we excluded participants with sleep duration between 8 and 9 hours (n=53) (Supplemental Table C-8). Analyses using 7 hours (n=399) as the cut point for short vs. longer sleep duration did not differ materially from analyses using 6 hours as the cut point but analyses using 5 hours (n for short sleepers =69) as the cut point differed in that point estimates were lower than those using 6 hours as the cut point and did not reach statistical significance for both the whole and restricted (<8 hour) samples (Supplemental Table C-9). For sleep efficiency, very few participants would have been categorized as having "low sleep efficiency" for cut points lower than 85%. Finally, in this analysis we could not demonstrate an interaction effect of gender, age or race/ethnicity on the relationship between

short sleep duration and/or poor sleep quality and diurnal cortisol.

Discussion

In this population-based study of adults, using multiple measures of salivary cortisol and objective in-home sleep data, we found that sleep duration and sleep quality were associated with alterations of the diurnal cortisol patterns. Notably, short sleep duration was associated with less pronounced late decline in cortisol and less pronounced wake-to-bed slope. These associations remained significant after adjustment for sleep apnea, sleep efficiency and other covariates and were robust to various sensitivity analyses. Low sleep efficiency was also associated with less pronounced early cortisol decline although was not confirmed in analyses of the slope of wake-to-bed cortisol. The association also remained significant after adjustment for sleep apnea, sleep duration and other covariates. In stratified analyses, we found some evidence that associations of sleep duration with diurnal salivary cortisol were stronger among study participants who also reported insomnia symptoms than among those who did not. The test for interaction was of marginal statistical significance. We did not find associations of sleep characteristics with AUC, suggesting that this summary measure may be less sensitive to sleep-associated alterations in the diurnal cortisol profile than specific components (features of the cortisol) examined using the piecewise linear mixed models.

Several observational studies have described alterations of the diurnal cortisol profile associated with psychological and psychosocial factors as well as with health outcomes. Although the findings are mixed, a consistent pattern that seems to be emerging is the association of adverse risk profiles (or disease outcomes) with a flatter decline in cortisol over the day (and consequent higher bedtime levels). For example, a flattened diurnal pattern has been associated with atherosclerosis in the CARDIA study;[144] and a flattened slope, due to lower morning cortisol, was associated with home-related stress.[145] Findings for the CAR, however, have been more mixed. For instance, higher CAR has been found in patients with depression compared to non-depressed persons,[105,106] but also a blunted CAR has been found in patients with depression compared to normal subjects.[124] A large CAR and flattened diurnal decline was found to be associated with work-related stress;[146] and a flattened CAR was also associated with burnout.[147] Lower cortisol levels at wake-up and less pronounced early decline in cortisol associated with low-socioeconomic

status,[136] low CAR levels associated with diabetes[126] and low levels of awakening cortisol and a less pronounced decline in cortisol associated with markers of obesity[127] have been found in the MESA Study.

We consistently found a flatter decline in cortisol associated with short sleep duration that was present in the overall population, those with and without insomnia, and after controlling for sleep efficiency, suggesting that short sleep duration independent of insomnia and poor sleep is associated with evening cortisol elevation. We observed associations with the late decline and the total wake to bed slope. The flatter late decline in cortisol seen in our population with short sleep duration could be attributable to either low awaking cortisol level or high evening cortisol level. However, we found no association of short sleep duration with awakening cortisol levels suggesting that changes in wake-up level did not account for these slope differences. The flattened evening cortisol associated with sleep duration found in our study could also be explained by the effect of age, but our analyses adjusted for age and we found same associations after restricting our sample to participants with ≤75 years old. It has been suggested that older people keep more a "typical" diurnal cortisol profile than younger people.[148]

Only a few population-based studies have examined associations of sleep with the daily cortisol profile. The Whitehall II Study,[80] a large cohort of London-based civil servants, with 2751 participants collected up to six salivary cortisol samples on a weekday, but sleep duration and sleep quality were self-reported. The study found that short sleep duration was associated with an increased CAR and a shallower slope in cortisol, and that sleep disturbance was associated with a shallower slope in cortisol. Our study differs from the Whitehall Study in having objective data of sleep including sleep duration, sleep efficiency and apnea, and up to 16 salivary samples per participant. Our findings are consistent with the Whitehall findings in that shorter sleep duration and poorer sleep efficiency were associated with a less pronounced decline in cortisol.

A second relevant study is a Danish study[81] (n~4,060) with salivary samples at three time points in a day and self-reported sleep duration and quality. This study did not find associations between sleep duration and cortisol but did find associations between sleep problems and lower morning cortisol. In a follow-up study with 387 participants, with one additional salivary sample (at awakening), the study also

found associations between poor sleep quality and less pronounced decline in cortisol. Our has analysis that was based on a larger number of salivary samples over two days, objective measures of sleep duration and quality, and a more heterogeneous population.

Other observational studies using different methodology from our study found no associations. A study from China[115] examined 96 study participants using 3-day actigraphy with 3 days of only awakening salivary cortisol and found that salivary awakening cortisol was not associated with sleep duration and sleep efficiency. This study was unable to characterize the diurnal cortisol profile. In the Osteoporotic Fractures in Men (MrOs) Study,[79] actigraphy-based sleep duration and 24-hour free urinary cortisol were measured. No association between sleep duration and urinary cortisol was found. Urinary cortisol has the advantage of assessing nighttime cortisol levels, but urinary free cortisol represents a small fraction (2−3%) of the total cortisol released by the adrenal cortex.[149] It is a summary measure and it does not characterize the diurnal cortisol profile.

Prior studies have not reported associations of sleep duration with cortisol stratified by insomnia symptoms**.** When we compared the association between sleep duration and salivary cortisol in participants with and without insomnia we found that associations of sleep duration with cortisol were stronger in those who reported insomnia. There was some evidence that in the group with insomnia shorter sleep duration was associated with a less pronounced CAR and a less pronounced late decline in cortisol compared to longer sleep duration. However, interactions between insomnia and sleep duration were not statistically significant in our sample. The stronger association of sleep duration with cortisol in those who reported insomnia could be related with the findings of the joint effect of short sleep duration and insomnia on hypertension,[33] type-2 diabetes,[31] and mortality[129] reported by Vgontzas et. al.

We also found that insomnia was associated with a reduced CAR in the full sample and this association was driven by the short sleepers whereas insomnia was associated with a more pronounced decline in the longer sleepers (persons who slept ≥6 hours). This finding is in part in accordance with a study in which higher indexes of sleep disturbance, in subjects with primary insomnia, were associated with lower awakening cortisol, and with non-significant lower CAR.[91] But, it is not in agreement with other

studies in which insomnia was associated with elevation of the evening cortisol,[130,131] or no association was found.[132] These mixed findings suggest that more studies need to be done in this area.

Our study has several limitations. First, our data are cross-sectional and therefore we cannot rule out bidirectional causality.[96,150] We approached the analysis a priori from a causal framework in which sleep duration and quality were considered as the independent variables and HPA axis measures were taken as the dependent variables but cannot determine the direction of causality (e.g. we cannot be sure that elevation of cortisol later in the day prevents a normal and/or good sleep vs. the other way around.) Second, both sleep and cortisol data were collected from each subject at a single point in time, and intra-individual variability over time for both sleep and cortisol profiles was not captured. However, sleep stability has been evaluated in at least two studies. In the CARDIA Study, investigators determined that even though night-to-night sleep is variable, sleep parameters (duration, efficiency, and latency) measured by actigraphy and supplemented by a log diary tend to be relatively stable from year to year in middle age adults.[151] A study among middle-aged Chinese adults reported similar findings over 3-year period.[115] The daily cortisol profile also varies from day to day. The use of two consecutive days is an advance over single day studies. Data from MESA suggest that the long term stability over 6 years is moderate (intraclass correlation 0.25 for the early decline and 0.42 for the late decline).[152] Variability in both sleep and cortisol over time may have resulted in underestimates of the true associations. Third, the definition of insomnia is dependent on the subjective experience of disturbed sleep,[153] but most importantly is a common clinically recognized entity that may represent several subphenotypes that need to be identified. Fourth, power to assess associations with poor sleep efficiency was limited because only 10% of our participants sample had low (<85%) sleep efficiency.

A strength of our study is the availability of high quality data for a variety of covariates including sleep apnea. Except for one study,[79] the studies mentioned above did not adjust for this variable. A second strength is that we had objective sleep data derived from 7-day actigraphy and 1-night PSG. A third strength is that we had up to 16 salivary samples over two days, allowing us to model the diurnal cortisol profile. A fourth strength is our analytic approach. We used piecewise linear mixed models with splines to account for the non-linear diurnal profile, for the correlations of the repeated cortisol measures

within individual, for varying number of observations, and for differences in the timing of data collection. A fifth strength is our large diverse population based sample.

In summary, our results provide evidence of associations of shorter sleep duration and worse sleep quality with alterations in features of the diurnal cortisol profile. These differences in the diurnal cortisol profile may contribute to the increased risk of cardiovascular disease, cardiovascular risk factors and other health outcomes that has been described in association with shorter sleep duration and poorer sleep quality.

Table 3-1 Characteristics of participants (n= 600) by sleep duration and sleep efficiency, MESA Study (2010-2012)

	Sleep Duration (hours)			**Sleep Efficiency** (%)		
	< 6 h (n = 202)	≥ 6 h (n = 398)	p-value	< 85% (n = 60)	≥ 85% (n = 540)	p-value
	N (%) or Mean ± SD			N (%) or Mean ± SD		
Demographics						
Age (years)	68.6 ± 9.0	69.4 ± 9.0	*0.3*	70.3 ± 7.4	69.0 ± 9.2	*0.2*
Male	106 (53)	178 (45)	*0.07*	35 (58)	249 (46)	*0.07*
Race/ethnicity			*<.001*			*0.2*
White	36 (18)	130 (33)		11 (18)	155 (29)	
Black	92 (45)	99 (25)		24 (40)	167 (31)	
Hispanic	74 (37)	169 (42)		25 (42)	218 (40)	
Income-Wealth Index			*0.1*			*0.03*
0 − 1	33 (18)	41 (12)		12 (21)	62 (13)	
2 − 3	41 (22)	83 (24)		5 (9)	119 (25)	
4 − 6	80 (43)	142 (41)		28 (49)	194 (41)	
7 − 8	33 (18)	83 (24)		12 (21)	104 (22)	
Married	98 (50)	230 (59)	*0.05*	35 (59)	293 (56)	*0.6*
Lifestyle characteristics						
Body Mass Index[1] kg/m^2			*<.001*			*0.2*
Normal	30 (15)	88 (22)		8 (13)	110 (20)	
Obesity Grade 1	75 (37)	163 (41)		24 (40)	214 (40)	
Obesity Grade 2	82 (41)	139 (35)		23 (38)	198 (37)	
Obesity Grade 3	15 (7)	8 (2)		5 (8)	18 (3)	
Smoking status			*0.1*			*0.3*
Current	23 (11)	25 (6)		4 (7)	44 (8)	
Former and quit > 1 year ago	66 (33)	156 (39)		28 (47)	194 (36)	
Never	112 (56)	215 (54)		28 (47)	299 (56)	
Alcohol consumption (Yes/No)	75 (37)	164 (41)	*0.4*	23 (38)	216 (40)	*0.8*
Hypertension	127 (63)	231 (58)	*0.3*	37 (62)	321 (59)	*0.7*
Diabetes	83 (41)	165 (42)	*0.9*	30 (50)	218 (41)	*0.2*
Depression CESD >16	39 (20)	58 (15)	*0.1*	11 (19)	86 (16)	*0.7*
Hostility (tertiles)			*0.2*			*0.9*
Highest	58 (29)	95 (25)		16 (27)	137 (26)	
Second	87 (44)	151 (40)		23 (39)	215 (41)	
Lowest	55 (28)	133 (35)		20 (34)	168 (32)	
Oral Steroids users	3 (2)	5 (1)	*0.8*	2 (3)	6 (1)	*0.2*
Inhaled Steroids users	11 (6)	4 (1)	*<.001*	2 (3)	13 (2)	*0.7*
Antihypertensive users	122 (60)	218 (55)	*0.2*	35 (58)	305 (57)	*0.8*
HRT[2] users	4 (2)	14 (4)	*0.3*	0 (0)	18 (3)	*0.2*
Antidepressant users	17 (8)	49 (12)	*0.2*	6 (10)	60 (11)	*0.8*

Sleep Variables						
Insomnia told by a doctor	14 (7)	27 (7)	*0.9*	*4 (7)*	*37 (7)*	*0.9*
Insomnia symptom [3]	75 (38)	134 (34)	*0.4*	24 (41)	185 (35)	*0.4*
Sleep efficiency (%)						
Low (<85%)	32 (16)	28 (7)	*<..001*	NA	NA	
Sleep Duration						
Short (<6 hours)	NA	NA		32 (53)	170 (32)	*<.001*
Apnea and hypopnea Index[4] (AHI) ≥ 15	109 (63)	214 (58)	*0.3*	*39 (72)*	*284 (52)*	*<0.05*
Cortisol Measures						
Median (Q1-Q3), (mmol/L)						
Wake-up	15.2 (10.0-20.5)	16.5 (11.4-24.4)	*0.02*	13.9 (7.1-19.8)	16.2 (11.2-22.9)	*0.02*
30 minutes later	19.7 (12.0-29.4)	23.7 (15.7-31.9)	*0.01*	17.4 (11.3-29.0)	22.9 (15.2-31.6)	*0.01*
1 hour (h) after breakfast	11.6 (8.0-16.5)	11.4 (8.4-16.4)	*>0.1*	11.7 (8.6-15.0)	11.4 (8.2-16.5)	*>0.1*
10:00 h or around	8.8 (6.2-12.3)	9.0 (6.6-13.1)	*>0.1*	8.9 (6.5-14.5)	9.0 (6.5-12.5)	*>0.1*
Noon or around	7.7 (5.0-11.3)	7.5 (5.3-10.5)	*>0.1*	7.0 (5.0-11.9)	7.6 (5.2-10.6)	*>0.1*
16:00 h or around	5.6 (3.7-9.1)	5.5 (3.6-8.2)	*>0.1*	5.7 (3.4-8.7)	5.5 (3.7-8.3)	*>0.1*
18:00 h or around	4.2 (2.8-6.6)	4.1 (2.6-6.6)	*>0.1*	4.3 (2.5-7.0)	4.1 (2.7-6.4)	*>0.1*
Bedtime	3.6 (2.1-7.0)	3.3 (1.9-5.3)	*0.03*	3.1 (1.8-5.5)	3.4 (2.0-5.8)	*>0.1*

*either chi-square or t-test/ANOVA or Kruskall Wallis test for variables non-normally distributed

(1) Body mass index, WHO categories:
   a. Normal: 18.5 – 24.9
   b. Obesity I : 30.0-34.9
   c. Obesity II: 35.0-39.9
   d. Obesity III: ≥ 40.0

(2) HRT = Hormone Replacement Therapy

(3) assessed by WHIIRS score ≥9 = insomnia

(4) apnea (all apneas and hypopnea per hour of sleep with ≥ 3% or greater desaturation – index (ahi))

Table 3-2. Characteristics of study participants by insomnia symptoms (n= 591) and overall, MESA Study (2010-2012)

	Insomnia Symptoms						P-value
	Yes		No		Total		
	N	%	N	%	N	%	
	209	35.4	382	64.6	591	100.0	
Demographics							
Age group							0.6
54 − 64	75	35.9	130	34.0	205	34.7	
65−74	68	32.5	139	36.4	207	35.0	
>75	66	31.6	113	29.6	179	30.3	
Male	79	37.8	200	52.4	279	47.2	**<.001**
Race/ethnicity							0.3
White	50	23.9	113	29.6	163	27.6	
Black	68	32.5	120	31.4	188	31.8	
Hispanic	91	43.5	149	39.0	240	40.6	
Income−wealth index							**<0.01**
0−1	32	15.3	41	10.7	73	12.4	
2−3	51	24.4	71	18.6	122	20.6	
4−6	75	35.9	144	37.7	219	37.1	
7−8	26	12.4	88	23.0	114	19.3	
Married	99	47.4	225	58.9	324	54.8	**0.02**
Lifestyle characteristics							
Body mass index							0.3
Normal	43	20.6	74	19.4	117	19.8	
Obesity grade 1	88	42.1	146	38.2	234	39.6	
Obesity grade 2	67	32.1	150	39.3	217	36.7	
Obesity grade 3	11	5.3	12	3.1	23	3.9	
Smoking status							0.8
Current	16	7.7	30	7.9	46	7.8	
Former (quit >1 year ago)	81	38.8	138	36.1	219	37.1	
Never	111	53.1	212	55.5	323	54.7	
Alcohol user	74	35.4	160	41.9	234	39.6	0.1
Hypertension	124	59.3	230	60.2	354	59.9	0.8
Diabetes	89	42.6	156	40.8	245	41.5	0.8
Depression CESD >16	51	24.4	44	11.5	95	16.1	**<.0001**
Hostility (tertiles)							**0.03**
Highest	60	28.7	90	23.6	150	25.4	
Middle	85	40.7	149	39.0	234	39.6	
Lowest	50	23.9	136	35.6	186	31.5	
Oral steroids user	2	1.0	6	1.6	8	1.4	0.5
Inhaled steroids user	4	1.9	1	0.3	5	0.8	0.5
Antihypertensive user	121	57.9	214	56.0	335	56.7	0.7
HRT user	5	2.4	13	3.4	18	3.0	0.5
Antidepressant user	24	11.5	42	11.0	66	11.2	0.9
Sleep variables							
Insomnia told by a doctor[1]	29	13.9	12	3.1	41	6.9	**<.0001**
Sleep duration < 6 hours	75	35.9	123	32.2	198	33.5	0.4
Sleep efficiency % <85%	24	11.5	35	9.2	59	10.0	0.4
Apnea/hypopnea index ≥ 15	111	53.1	77	20.2	188	31.8	0.8

	Mean	SD	Mean	SD	Mean	SD	p−value
Wakeup after sleep onset[2] (min)	43.7	17.8	40.3	15.5	41.5	16.5	**0.02**
Sleep latency[3] (min)	4.1	2.4	4.4	2.7	4.2	2.6	0.1
Cortisol measures	**Median**	**Q1−Q3**	**Median**	**Q1−Q3**	**Median**	**Q1−Q3**	**p−value**
Wake−up	16.3	11.0−23.3	16.0	10.8−22.6	16.1	10.9−22.7	>0.1
30 minutes later	21.3	13.6−29.6	22.3	15.4−32.0	22	14.7−30.9	0.08
1 hour after breakfast	11.8	8.5−16.5	11.3	8.0−16.3	11.4	8.2−16.5	>0.1
~10:00 am	8.9	6.6−12.4	8.9	6.5−12.9	8.9	6.5−12.6	>0.1
~ Noon	7.5	5.2−10.6	7.6	5.2−10.8	7.5	5.2−10.7	>0.1
~ 16:00 (4:00 pm)	5.3	3.5−8.5	5.8	3.7−8.2	5.5	3.7−8.3	>0.1
~ 18:00 (6:00 pm)	4.0	2.6−6.5	4.3	2.7−6.6	4.1	2.6−6.6	>0.1
Bedtime	3.3	1.9−6.1	3.3	2.0−5.7	3.3	2.0−5.8	>0.1

*either chi-square or t-test/ANOVA or Kruskall Wallis test for variables non-normally distributed

(1) insomnia disorder

(2) WASO, wake time after sleep onset, an indicator of sleep fragmentation

(3) period between bed time and sleep onset

Table 3-3.Percent differences (95% confidence intervals) in features of the daily cortisol (wake-up, CAR, early decline and late decline) and summary measures of cortisol (wake-to-bed slope and AUC) associated with sleep duration and sleep efficiency

	Sleep Duration[a]	**Sleep Efficiency**[b]
	(< 6 hr vs.≥ 6 to < 9 hr)	(< 85% vs.≥ 85%)
Percent difference in wake-up		
Model 1[c]	−11.93 (−30.9, 4.3)	−33.73 (−81.5, 1.5)
Model 2[d]	−12.57 (−34.4, 5.7)	−37.91 (−93.8, 1.8)
Model 3[e]	−10.95 (−33.0, 7.4)	−39.44 (−97.4, 1.5)
Model 4[f]	−8.20 (−29.1, 9.3)	−36.98 (−93.5, 3.0)
Percent difference in cortisol awakening response (CAR)		
Model 1[c]	−8.70 (−25.2, 5.6)	−2.00 (−27.6, 18.47)
Model 2[d]	−8.16 (−26.6, 7.6)	0.29 (−21.9, 28.7)
Model 3[e]	−9.40 (−28.5, 6.9)	0.09 (−22.2, 28.8)
Model 4[f]	−9.57 (−27.9, 6.1)	2.35 (−20.1, 31.0)
Percent difference in early decline		
Model 1[c]	6.34 (−5.0, 19.0)	**22.12 (0.7, 48.2)**[§]
Model 2[d]	6.02 (−6.4, 20.0)	**25.97 (2.8, 54.4)**[§]
Model 3[e]	5.28 (−7.3, 19.6)	**27.65 (3.7, 57.2)**[§]
Model 4[f]	2.35 (−9.7, 15.9)	**28.96 (4.1, 59.7)**[§]
Percent difference in late decline		
Model 1[c]	**1.86 (0.6, 3.1)**[*]	−0.85 (−2.7, 1.0)
Model 2[d]	**2.02 (0.7, 3.4)**[*]	−1.05 (−3.2, 1.0)
Model 3[e]	**2.05 (0.7, 3.5)**[*]	−0.98 (−3.1, 1.1)
Model 4[f]	**2.23 (0.8, 3.7)**[*]	−1.84 (−4.2, 0.4)
Summary measures of cortisol		
Percent difference in wake-to-bed slope		
Model 1[c]	**2.15 (1.1, 3.2)**[***]	0.45 (−1.2, 2.1)
Model 2[d]	**2.33 (1.2, 3.5)**[***]	0.59 (−1.2, 2.4)
Model 3[e]	**2.27 (1.1, 3.5)**[***]	0.75 (−1.0, 2. 6)
Model 4[f]	**2.22 (1.0, 3.4)**[**]	0.06 (−1.8, 1.9)
Percent difference in area under the curve (AUC)		
Model 1[c]	0.34 (−10.6, 12.7)	−8.97 (−32.6, 10.5)
Model 2[d]	0.77 (−11.4, 14.9)	−6.56 (−32.8, 14.5)
Model 3[e]	0.28 (−12.1, 14.5)	−5.51 (−32.2, 15.8)
Model 4[f]	−0.15 (14.6, 12.5)	−5.28 (−32.8, 16.6)

[§]<0.05; [*]≤0.01; [**]≤0.001 ; [***]≤0.0001

[a] Sleep duration was estimated as dichotomized (short sleep duration (3 to < 6 hours)) vs. longer (reference, ≥ 6 hours to < 9hours)

[b] Sleep efficiency was estimated as dichotomized (low sleep efficiency (< 85%)) vs. high (reference, ≥ 85%)

Models:

[c] Model 1 : Model adjusted for day of salivary collection, time of wake-up, gender, age, race/ethnicity, and income-wealth index

[d] Model 2 : Model 1 + body mass index, smoking, alcohol consumption, medications (oral and inhaled steroids, hormone replacement therapy and antidepressants) and apnea/hypopnea index (AHI)

[e] Model 3 : Model 2 + hypertension, diabetes, and depression

[f] Model 4 : Model 3 + sleep efficiency (as continuous) or sleep duration (as continuous) in models for sleep duration and sleep efficiency, respectively

Table 3-4. Percent differences (95% C.I.) in features of the daily cortisol and summary measures of cortisol associated with insomnia and stratified by short and longer sleepers

	Insomnia[a] vs. non-insomnia		
	Overall sample (3 h to < 9 h)	Short Sleepers[b] < 6 hours	Longer Sleepers[b] ≥ 6 hours
Percent difference in wake-up			
Model 1[c]	8.28 (−5.9, 24.7)	5.43 (−17.5, 34.8)	11.17 (−6.6, 32.3)
Model 2[d]	6.40 (−8.7, 24.0)	9.40 (−17.5, 45.1)	7.10 (−10.7, 28.5)
Model 3[e]	5.70 (−9.5, 23.5)	7.63 (−19.4, 43.8)	7.85 (−10.1, 29.3)
Model 4[f]	6.97 (−8.6, 25.2)	10.86 (−16.9, 47.9)	8.18 (−10.0, 30.0)
Percent difference in cortisol awakening response (CAR)			
Model 1[c]	**−18.73 (−36.1, −3.5)***	**−38.86 (−77.5, −8.6)***	−8.30 (−27.2, 7.8)
Model 2[d]	**−16.43 (−35.0, −0.4)§**	**−33.40 (−76.17, −1.0)§**	−7.12 (−27.2, 9.8)
Model 3[e]	−15.90 (−34.8, 0.3)	**−36.53 (−80.5, −3.3)§**	−5.90 (−25.2, 10.4)
Model 4[f]	**−16.11 (−34.6, −0.1)§**	**−37.74 (−79.4, −5.7)§**	−5.90 (−25.4, 10.6)
Percent difference in early decline			
Model 1[c]	**11.44 (0.7, 23.3)§**	15.85 (−4.6, 40.7)	9.75 (−2.3, 23.3)
Model 2[d]	9.87 (−1.17, 22.2)	9.90 (−11.5, 36.4)	9.76 (−2.7, 23.8)
Model 3[e]	8.65 (−2.7, 21.3)	9.31 (−12.5, 36.5)	8.80 (−4.1, 21.8)
Model 4[f]	7.52 (-3.5, 19.8)	6.12 (-13.9, 30.9)	7.80 (−4.3, 21.5)
Percent difference in late decline			
Model 1[c]	−1.05 (−2.3, 0.2)	0.22 (−1.9, 2.4)	**−2.02 (−3.4, −0.7)***
Model 2[d]	−1.06 (−2.4, 0.2)	0.35 (−2.0, 2.7)	**−2.01 (−3.5, −0.7)***
Model 3[e]	−1.10 (−2.5, 0.2)	0.53 (−1.8, 2.9)	**−2.19 (−3.7, −0.7)***
Model 4[f]	−1.09 (−2.4, 0.2)	0.91 (−1.4, 3.3)	**−2.27 (−3.7, −0.8)***
Summary measures			
Percent difference in wake-to-bed slope			
Model 1[c]	−0.60 (−1.6, 0.4)	0.17 (−1.7, 2.1)	**−1.20 (−2.5, −0.1)§**
Model 2[d]	−0.67 (−1.8, 0.4)	−0.07 (−2.2, 2.0)	**−1.20 (−2.3, −0.1)§**
Model 3[e]	−0. 78 (−1.9, 0.3)	0.07 (−2.0, 2.2)	**−1.38 (−2.5, −0.3)***
Model 4[f]	−0.86 (−2.0, 0.3)	0.14 (−2.0, 2.3)	**−1.52 (−2.6, −0.4)***
Percent difference in area under the curve (AUC)			
Model 1[c]	−0.38 (−11.4, 9.6)	−5.45 (−30.2, 14.6)	3.40 (−8.0, 16.2)
Model 2[d]	−2.26 (−14.4, 8.6)	−4.31 (−30.9, 16.9)	0.47 (−11.1, 13.6)
Model 3[e]	−4.43 (−17.3, 7.0)	−8.07 (−35.7, 13.9)	−0.60 (−14.3, 11.5)
Model 4[f]	−4.66 (−17.7, 6.9)	−7.77 (−35.5, 14.3)	−1.17 (−15.2, 11.2)

§ <0.05 *0≤0.01

[a] Insomnia symptoms WHIIRS (insomnia (score ≥ 9) vs. non-insomnia (reference, score < 9))

[b] Sleep duration was estimated as dichotomized (short sleep duration (3 to < 6 hours)) vs. longer (reference, ≥ 6 hours to < 9 hours)

[c] Model 1 : Model adjusted for day of salivary collection, time of wake-up, gender, age, race/ethnicity and income-wealth index

[d] Model 2 : Model 1 + body mass index, smoking, alcohol consumption, and medications (oral and inhaled steroids, hormone replacement therapy and antidepressants), and apneas and hypopneas index (ahi)

[e] Model 3 : Model 2 + hypertension, diabetes, and depression

[f] Model 4 (for insomnia) : Model 3 + sleep efficiency

Table 3-5. Percent differences (95% confidence intervals) in features of the daily cortisol and summary measures of cortisol associated with sleep duration specified as dichotomized and continuous stratified by insomnia symptoms

	Sleep Duration[b] (dichotomized) (reference, ≥ 6 hours of sleep)		Sleep Duration[b] (continuous) (1 hour less of sleep)	
	With Insomnia	Without Insomnia	With Insomnia	Without insomnia
Percent difference in wake-up				
Model 1[c]	−13.43 (−38.7, 7.2)	−10.65 (−37.6, 11.0)	−6.58 (−16.6, 2.6)	−4.52 (−14.2, 4.3)
Model 2[d]	−14.49 (−40.5, 6.7)	−15.93 (−48.1, 9.2)	−6.53 (−16.4, 2.5)	−4.31 (−15.2, 5.6)
Model 3[e]	−10.21 (−36.0, 10.7)	−14.34 (−46.9, 11.0)	−5.83 (−15.6, 3.1)	−3.39 (−14.6, 6.7)
Model 4[f]	−8.39 (−34.1, 12.4)	−10.71 (−41.5, 13.4)	−5.00 (−14.7,3.9)	−1.24 (−11.8, 8.4)
Percent difference in cortisol awakening response (CAR)				
Model 1[c]	-28.43 (-68.4, 2.0)	0.97 (−13.9, 18.4)	−10.43 (−25.2, 2.6)	1.15 (−6.2, 9.0)
Model 2[d]	-26.65 (-71.7, 6.6)	2.21 (−14.6, 22.4)	−9.44 (−24.6, 3.9)	2.32 (−5.7, 11.1)
Model 3[e]	**−36.89 (−85.5, −1.0)**[§]	2.98 (−13.9, 23.2)	−12.68 (−29.0, 1.5)	2.53 (−5.6, 11.4)
Model 4[f]	**−31.93 (−71.5, −1.5)**[§]	0.16 (−16.9, 20.8)	−10.52 (−23.6, 1.2)	0.87 (−7.4, 9.8)
Percent difference in early decline				
Model 1[c]	5.48 (−13.5, 28.7)	3.89 (−9.1, 19.3)	3.94 (−4.4, 13.0)	−0.04 (−8.1, 7.4)
Model 2[d]	3.70(−16.5, 28.8)	6.12 (−9.0, 23.7)	2.84 (−6.2, 12.8)	−1.26 (−9.7, 6.5)
Model 3[e]	5.65 (−15.7, 32.5)	5.12 (−9.7, 22.4)	2.64 (−7.2, 13.5)	−2.06 (−10.6, 5.8)
Model 4[f]	2.29 (−16.6, 25.5)	3.11 (−11.8, 20.6)	0.80 (−8.0, 10.5)	−3.59 (−12.8, 4.9)
Percent difference in late decline				
Model 1[c]	**3.39 (1.2, 5.6)**[**]	1.37 (−0.2, 2.9)	**1.11 (0.3, 2.0)**[*]	**0.87 (0.2, 1.6)**[*]
Model 2[d]	**3.53 (1.3, 5.8)**[*]	1.37 (−0.3, 3.1)	**1.17 (0.3, 2.0)**[*]	**0.94 (0.2, 1.7)**[*]
Model 3[e]	**3.68 (1.4, 6.0)**[*]	1.30 (−0.4, 3.0)	**1.32 (0.4, 2.2)**[*]	**0.98 (0.3, 1.7)**[*]
Model 4[f]	**3.55 (1.2, 6.0)**[*]	1.61 (−0.1, 3.4)	**1.26 (0.3, 2.2)**[*]	**1.22 (0.5, 2.0)**[*]
Summary measures of cortisol				
Percent difference in wake-to-bed slope				
Model 1[c]	**2.93 (1.0, 4.9)**[*]	**1.82 (0.5, 3.1)**[*]	**1.12 (0.3, 1.9)**[*]	**0.92 (0.3, 1.5)**[*]
Model 2[d]	**2.98 (1.1, 4.9)**[**]	**2.07 (0.7, 3.5)**[*]	**1.04 (0.3, 1.8)**[*]	**0.93 (0.3, 1.6)**[*]
Model 3[e]	**3.05 (1.0, 5.1)**[*]	**1.96 (0.6, 3.4)**[*]	**1.07 (0.2, 1.9)**[*]	**0.91 (0.3, 1.5)**[*]
Model 4[f]	**2.85 (1.0, 5.0)**[*]	**2.02 (0.6, 3.5)**[*]	**1.00 (0.1, 1.9)**[*]	**1.00 (0.3, 1.6)**[*]
Percent difference in area under the curve (AUC)				
Model 1[c]	−9.87 (−31.4, 8.1)	4.54 (−10.4, 22.0)	−4.11 (−13.4, 4.4)	1.97 (−4.3, 8.6)
Model 2[d]	−11.12 (−33.8, 7.7)	4.00 (−12.5, 23.6)	−4.27 (−13.2, 3.9)	2.04 (−4.7, 9.3)
Model 3[e]	−11.51 (−34.8, 7.7)	4.43 (−12.2, 24.2)	−5.97 (−15.0, 2.4)	2.30 (−4.6, 9.6)
Model 4[f]	−11.40 (−34.9, 8.0)	4.10 (−13.0, 24.5)	−6.12 (−15.3, 2.4)	2.18 (−5.0, 10.0)

[§]<0.05; [*]≤0.01; [**]≤0.001

[a] Insomnia symptoms (WHIIRS), dichotomized as with insomnia (score ≥ 9) and without insomnia

[b] Sleep duration was estimated as continuous (hours) and dichotomized (short sleep duration (<6hours)) vs. longer (reference, ≥ 6hours to < 9hours)

Models:

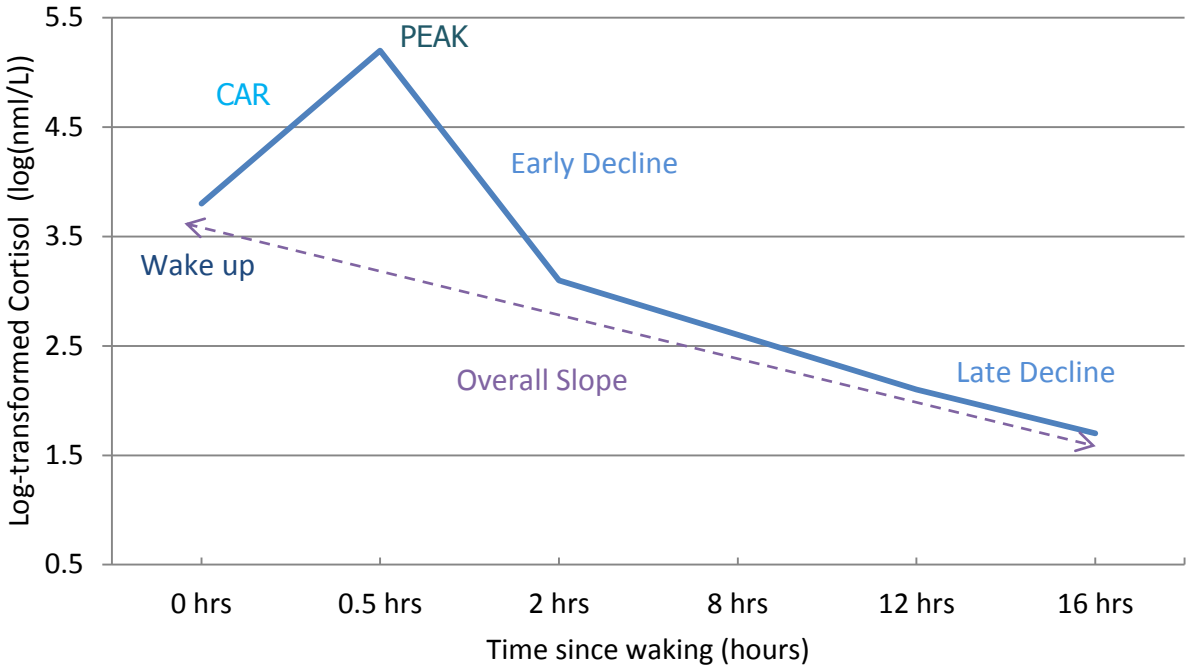
[c] Model 1 : Model adjusted for day of salivary collection, time of wake-up, gender, age, race/ethnicity, and income-wealth index

[d] Model 2 : Model 1 + body mass index, smoking, alcohol consumption, medications (oral and inhaled steroids, hormone replacement therapy and antidepressants) and apnea/hypopnea index (AHI)

[e] Model 3 : Model 2 + hypertension, diabetes, and depression

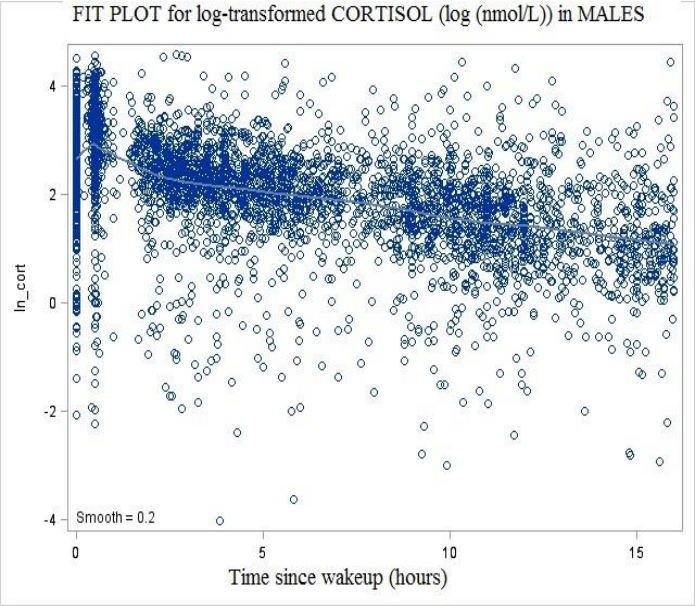
[f] Model 4 : Model 3 + sleep efficiency (as continuous)

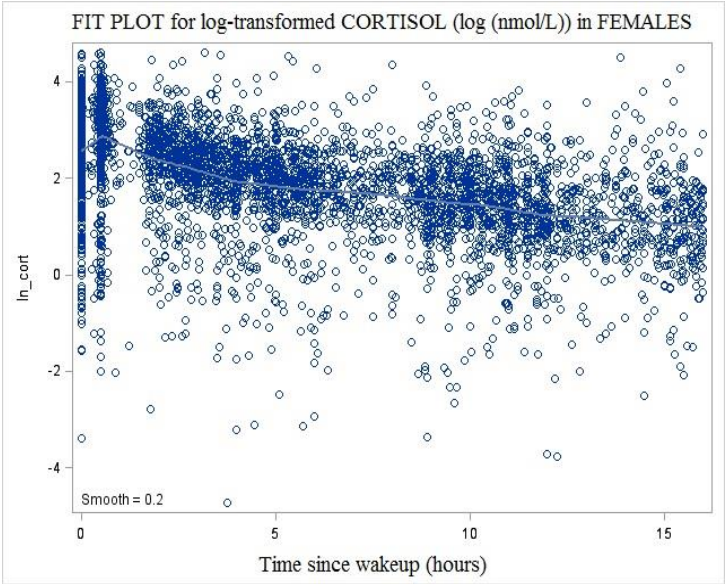
Figure 3-1. Features of the diurnal cortisol profile



(CAR = Cortisol awakening response)

Figure 3-2. LOESS plot of the cortisol daily profile for male and women





4. CHAPTER 4. SLEEP DURATION AND QUALITY AND HORMONAL AND CARDIOVASCULAR RESPONSES TO A STRESS CHALLENGE PROTOCOL

Abstract

Context:

Short sleep duration and poor sleep quality are associated with adverse cardiovascular outcomes. Potential mechanisms include alterations in autonomic nervous system (ANS) activity and dysfunction of the hypothalamic-pituitary adrenocortical (HPA) axis.

Objective:

To examine the association of sleep duration and/or sleep quality and cardiovascular and hormonal responses to a stress challenge protocol.

Design:

Data from the Multi-Ethnic Study of Atherosclerosis (MESA) including actigraphy-based measures of sleep duration and efficiency and cardiovascular (heart rate (HR) and HR variability (HRV)) and hormonal (amylase and cortisol) responses to a stress challenge protocol were obtained from 527 participants ages 54-93 years at MESA Exam 5 (2010-2012).

Results:

Neither sleep duration nor sleep efficiency were associated with exaggerated heart rate responses to challenge. Participants with low sleep efficiency had lower levels of high frequency heart rate variability (HF-HRV) at baseline than those with high sleep efficiency (fully adjusted model −0.58, 95% CI −1.03 to −0.14) but there was no association between sleep efficiency and HF-HRV responses to challenge. Study participants who reported insomnia had greater HF-HRV orthostatic reactivity (fully adjusted model −0.22; 95% CI −0.444 to −0.002) than those who did not. Short sleep duration, low sleep efficiency and insomnia were not associated with salivary amylase or cortisol responses to challenge.

Conclusions:

In a population based sample, insomnia symptoms were associated with greater high frequency heart rate variability in response to orthostatic stress challenge.

Introduction

Short sleep duration has been found in epidemiological studies to be associated with higher risk of coronary heart disease (CHD),[8,18] subclinical cardiovascular disease (CVD),[19,20] and CVD risk factors.[13] Poor sleep quality has also been found associated with CVD outcomes. Sleep difficulty was associated with CHD mortality in men;[121] and insomnia symptoms predicted increased risk for acute myocardial infarct in a large cohort of men and women[26] and CVD mortality in men.[120] Objective poor sleep quality as indexed by low sleep efficiency was also associated with elevated blood pressure among adolescents.[30]

Studies have also linked cardiovascular reactivity/recovery to stressors to cardiovascular-related outcomes. Exaggerated blood pressure reactivity to mental stress predicted hypertension in adult males,[154] coronary artery calcium in young subjects[42] and carotid atherosclerosis in middle age adults.[155] Delayed blood pressure recovery from mental stress has also been associated with carotid atherosclerosis.[156]

Several biological pathways for the associations between sleep loss and CVD have been suggested including dysfunction of the hypothalamic pituitary-adrenal axis (HPA) and autonomic nervous system (ANS). These systems interact and reinforce each other in response to emotional stress.[38,39] Stress system metabolites are higher during arousal and during the day and lower during sleep. For example, among young volunteers, evening plasma cortisol levels were higher after a whole[47] and partial night[48] of sleep deprivation than after a normal night of sleep. Plasma norepinephrine (NE) and epinephrine (E) were lower during sleep than during wakefulness[61] and higher during nighttime hours when subjects were awakened than at other hours.[62] Heart rate and blood pressure were lower during slow wave sleep (SWS) than during wakefulness.[63]

Cardiovascular responses to the mental stress challenge test

The sympathetic and parasympathetic branches of the ANS interact with each other[157] such as health may be affected when the parasympathetic cannot modify the excessive activity of the sympathetic system.[158] . High frequency heart rate variability (HF-HRV) is an accepted measure of parasympathetic tone.[159] Experimental studies have also evaluated ANS markers during normal sleep and during sleep deprivation. For example, heart rate (HR) is slower during deep sleep than during wakefulness.[160] In a

sample with 338 healthy adults, those who slept <6 hours/night had higher HR when awake than those who slept ≥6 hours.[161] After 32 hours of sleep deprivation, healthy young men had higher blood pressure and HR and elevated normalized low-frequency HRV than without sleep deprivation.[162] Insomnia was associated with decreased high/total frequency spectral power and increased low/high frequency spectral power in one study[65] and SWS deprivation[163] have also been associated with reduced normalized high frequency HRV. To our knowledge, the associations of reduced sleep duration and/or sleep quality and stress-induced HR/HRV responses have only been investigated in restrictive population samples. In studies of small samples of healthy young volunteers, the effect of sleep deprivation on subsequent HR/HRV responses to a mental stress has been investigated.[67,68,109] However, these studies have inconsistent findings.

Hormonal responses to the mental stress challenge test

Salivary alpha-amylase (sAA), a marker of the sympathetic branch of the ANS, has also been studied in psychophysiological research.[71,164] Amylase increases immediately after stress and returns to baseline levels within 10-20 minutes.[75] Associations have been reported between sAA reactivity to mental and physical stress and plasma norepinephrine,[73] shortened cardiac pre-ejection period.[74] sAA reactivity was also correlated with heart rate (HR) and with low-frequency HRV/high frequency HRV ratio, which reflects sympathovagal balance.[73,75] A few studies have examined the association of sleep with sAA responses to mental stress, but these have been restricted to children[165] or healthy young adults.[101] No reported study to our knowledge has evaluated the association of habitual sleep characteristics with sAA mental reactivity and/or recovery in a population-based sample.

Diurnal cortisol patterns are markers of the HPA, and cortisol secretion can also increase in response to acute mental stress (probably due to pulsatility cortisol).[166] Attenuated cortisol reactivity to mental stress has also been found in some circumstances such as in smokers[167] and depression.[168] Animal studies have found association between sleep alterations and cortisol responses to stress.[169] This association has also been investigated in human but only in restricted population samples such as children[170] and women.[89]

Despite evidence linking sleep to sympathetic and parasympathetic activity as well as stress reactivity to cardiovascular outcomes, only a few small experimental studies have examined how sleep affects cardiovascular reactivity to a stress challenge. Some have found associations between sleep deprivation and autonomic alterations[67,68,109] but others did not.[69] To our knowledge, only one study has evaluated the effect of habitual short sleep duration on subsequent HR and HRV responses to a stress challenge,[87] but this study was restricted to a small sample of young adults. Indeed, there is lack of studies of sleep alterations and hormonal responses to a stress challenge.

Aims of the study

Establishing a link between short and/or poor sleep and altered cardiovascular and hormonal responses to a stress challenge is important to establishing whether sympathetic/parasympathetic alterations are part of the pathophysiologic mechanisms linking sleep to subsequent cardiovascular events.

We hypothesized that: (1) participants whose sleep was short or of poor quality would have higher level heart rate (HR) and higher value of amylase and cortisol at baseline and lower level high-frequency heart rate variability (HF-HRV) at baseline than individuals who slept longer or better; (2) participants whose sleep was short or of poor quality would have greater HR, greater amylase and cortisol reactivity and reduced HF-HRV reactivity to mental and orthostatic stress challenges than individuals who slept longer or better; (3) participants whose sleep was short or of poor quality would have slower HR, HF-HRV and amylase and cortisol recovery from stress to baseline than individuals who slept longer or better.

Methods

Participants

The Multi-Ethnic Study of Atherosclerosis (MESA) is a longitudinal study designed to investigate risk factors for subclinical CVD. In 2000-2002, MESA recruited 6814 men and women to MESA Exam 1. At recruitment, participants were 45-84 years old, free of clinical CVD, and included six US communities. MESA was approved by the IRB at each field center, and all participants gave written informed consent at each exam. Our sample is a subset of MESA study participants, those from the Columbia, UCLA, and

John Hopkins field centers who completed the challenge test protocol (Stress study) and sleep assessments (Sleep study) during MESA Exam 5 in 2010-2012.

The Stress study excluded participants with night shift jobs. The Sleep study excluded participants that used oral airways support devices, nocturnal oxygen or nightly CPAP. We also excluded participants with <3 hours/night (n= 11) and participants with ≥9 hours/night (n= 11) average sleep duration from analysis.

A total of 1119 participants were enrolled in the cardiovascular (HR, HRV indices) part of the challenge test. We excluded 230 participants because of missing HR data at all 4 measurements in the protocol, failure to complete either mental stress tasks, or colorblindness. We excluded 362 additional participants who did not have actigraphy data. Our final analytic data set was based on 527 persons with 3450 HR/HRV measures (See Figure 4-1).

In the hormonal (amylase and cortisol) part of the challenge test, a total of 1040 participants were enrolled, providing 4040 salivary samples. Before we merged the stress data to the sleep data, we followed a few exclusion criteria on the cortisol data. We excluded participants that were missing all 4 saliva samples for the entire challenge test, individual samples with cortisol values of 0 nmol/L or over 100 nmol/L, participants who were blind color. The final sample before merging included 992 participants. From this group, 420 participants did not have actigraphy data; therefore, they were excluded reducing our sample to 572 persons with 2242 salivary samples. We further excluded participants who were using steroids and/or hormone replacement therapy (n=40). The final analytic data for cortisol was 532 persons with 2085 salivary samples. For amylase, we excluded participants who were missing all 4 saliva amylase values (n=18) or whose values were in the top 1% of the baseline sample (n=10 observations). We then excluded participants who were color blind or failed to complete both mental tasks (n=35), or who did not have actigraphy data (n=424). We further excluded participants who used betablockers (n=104) and/or oral steroids (n=5). For amylase the final analytic data set was based on 454 persons with 1736 amylase measures.

Sleep Protocol

The MESA Sleep protocol included 7-day actigraphy (Actiwatch Spectrum, Philips Respironics, Murrysville, PA), a sleep diary, and a sleep questionnaire. Actigraphic data during 30-second epochs were scored as sleep or wake by Actiware-Sleep®v.5.59 analysis software (Mini Mitter Co., Inc.). A validated algorithm[134] was used to calculate the activity count for each epoch. Intra-scorer intraclass correlation coefficients for average sleep duration and sleep efficiency were 0.91 and 0.97.

Sleep variables were averaged across all nights that the actigraph was worn. *Sleep duration* was defined as the average duration of sleep between sleep onset (sleep start time) and morning wakening (sleep end time) while in bed after "lights off." It was calculated by dividing the sum of the recorded minutes of sleep by the total number of main sleep periods. Sleep duration <6 hours was defined as "short" and ≥6 hours as "longer," consistent with previous studies. Sleep duration was also analyzed as at cut point of 7 hours. *Sleep efficiency* was defined as the percentage of time in bed after "lights off" spent sleeping. It was calculated by dividing the sum of sleep minutes by the sum of minutes after "lights off" during main sleep intervals recorded, and multiplying by 100. Sleep efficiency was categorized as "low" (<85%) or "high" (≥85%) consistent with previous studies. Insomnia, a subjective measure of sleep quality, was assessed based on self-report using the Women's Health Insomnia Rating Scale (WHIIRS), a 5-item questionnaire design to evaluate insomnia symptoms. The summary score ranged from 0 to 20. A score of ≥9 is considered clinically significant insomnia.[135] Naps were assessed from the actigraphy data and defined as the average sleep time in naps per day across all days when only naps of ≥15 minutes duration were counted.

Stress Challenge Protocol (Mental and Orthostatic Stress)

Participants were asked to minimize physical exercise during the hour preceding the test and not to take large meals, drink coffee, or smoke. After arriving at the laboratory and receiving instructions, each participant was connected to physiological monitors. Participants were instructed to remain silent throughout the protocol.

Two psychological stress tasks were administered via computer during a standard laboratory psychophysiology protocol while participants were in the seated position. For both tasks, participants

were told their performance on the tasks would be evaluated for both speed and accuracy. The protocol began with a resting baseline (11 minutes) followed by two mental stress tasks (6 minutes each) and a corresponding recovery period after each task (6 minutes each).

Mental stressors: Tasks were presented on a computer monitor; participants entered responses on a numeric keypad with their dominant hand. Tasks administration timing was controlled and synchronized to the physiological data acquisition via microcomputer. The order of the two tasks was varied randomly among participants and it was counterbalanced between subjects to minimize order effects. The Morgan and Turner Hewitt (MATH) task[57] involved the presentation on a computer screen for 1.5 seconds of pairs of numbers to be added or subtracted. Then, the word "equals" for 1.0 sec; followed by a possible answer to the problem for 1.0 sec, during which the participant used a keypad to indicate whether the presented solution was correct or not. Problems were ranked along five difficulty levels; response accuracy on each trial adjusted the difficulty of the next trial. A modified version of the Stroop color-word conflict task involved the presentation of one of four-color name words (blue, green, yellow or red) on a screen in a font color that either did or did not match the color name. When the color name stimulus appeared on the screen, the participant had to press the key on a keypad corresponding to the color of the font. To standardize the level of engagement, the presentation rate increased with better performance and decreased with poorer performance.

Following the mental tasks and their recovery periods, participants completed a 6-minute physical challenge test (orthostatic stress) that consisted of assuming and holding a standing position. This last period was followed by a 30-minute recovery period. Participants were continuously monitored by electrocardiogram (ECG) to collect HR and HRV data throughout the protocol (See Figure 4-2).

Heart rate (HR) and HR Variability (HRV)

Continuous measures of ECG were recorded during each period in the protocol. ECG electrodes were placed on the right shoulder, the left anterior axillary line at the 10th intercostal space, and the right lower quadrant. Analog ECG signals were digitized at 500 Hz by a Datacq9 cardiopulmonary monitor (Medelex, Inc., New York, NY) and passed to a microcomputer for R-wave detection implemented by

custom-written software (Graphical Acquisition, and Marking; Author: Delano MacFarlane, Ph.D.), resulting in an RR interval time series. Errors in marking of R-waves were corrected interactively.[171,172]

Heart Rate Variability (HRV). Mean HR and the standard deviation of the RR interval (SDRR), the root mean squared successive difference (rMSSD), and spectral power in the low (0.04-0.15 Hz (LF)) and high (0.15-0.40 Hz (HF)) frequency bands were computed from 5-min epochs using an interval method for computing Fourier transforms.[173] Prior to analysis, the RR interval was filtered using a Hanning window,[174] and power in the LF and HF bands is summed and adjusted for attenuation produced by the filter.[174] Time and frequency-domain HRV indices were expressed in ms and ms^2 respectively, and were log-transformed to achieve normal distribution.

For this dissertation, we will focus only in one of the HRV indices, the high-frequency HRV (HF-HRV) because this index has clearly been showed to reflect the parasympathetic function of the ANS whereas low-frequency HRV interpretation is still not clear. Besides, the time-domain HRV indices were highly correlated to HF-HRV.

Saliva collection

Four salivary samples were collected using a cotton oral swab during the challenge protocol: after a resting period at baseline, which was approximately 13 minutes since participants entered the exam (Sample #1); immediately after the completion of the mental tasks and their respective recovery periods (Sample #2), around 38 min after Sample # 1; after completion of the orthostatic stress (Sample #3) – around 17 min after Sample # 2, and after the 30-minute recovery period at the end of the stress challenge protocol (Sample #4) – around 30 min after Sample 3.

Salivary samples were stored at -20^0 C until analyses. After thawing, salivettes were centrifuged at 3,000 rpm for 5 min. Salivary cortisol level was determined using a commercially available chemiluminescence assay with high sensitivity (0.16 ng/mL) (IBL-Hamburg, Hamburg, Germany). Intra- and inter-assay coefficients of variation for the assay were <8%. Concentrations of alpha-amylase in saliva were measured using an enzyme kinetic method.[164] Intra- and inter-variability coefficient of variation for the amylase of the assay were <8% and <12%.

Covariates

Covariates used for for analysis of HR and HRV

Models of the association of sleep with HR/HRV were adjusted for factors known to impact heart rate,[175] specifically age, gender, race/ethnicity,(African American, Hispanic, or white, income-wealth index, body mass index (BMI, kg/m^2), smoking status and alcohol consumption as of MESA Exam 5. Smoking status was categorized as current and recent smoker (quit less than a year ago), past smoker (quit more than a year ago), or never smoker. Alcohol consumption was classified as yes/no. Other covariates were diabetes and use of medications. Diabetes mellitus status was categorized according to the 2003 criteria of the American Diabetes Association as normal, impaired fasting glucose, untreated diabetes, and treated diabetes. We also classified diabetes status as no (normal) vs. yes (otherwise). Use of medications was categorized as yes if the participant used anti-hypertensive medications, medications for sleep and mood or sympathomimetic medications or no if the participant did not use those specific medications. Analyses were also adjusted for sleep efficiency when the main predictor was sleep duration and for sleep duration when sleep efficiency was the main predictor. Additionally, we adjusted for the apnea hypopnea index (AHI), including only apneas and hypopneas associated with ≥3% desaturation, and for naps defined as the average sleep time in naps per day across all days in the recording counting only naps with 15 minutes or more of sleep time. We did not adjust for hypertension but we adjusted for use of anti-hypertensive medication.

Covariates used for analysis of amylase and cortisol

For amylase, analysis involved the same covariates as above. Use of medications also included hormone replacement therapy and inhaled steroids. Additionally, we adjusted for sleep duration or sleep efficiency, and for the apnea hypopnea index (AHI).

Statistical Analyses

We examined participant characteristics by categories of sleep duration and efficiency. Differences in outcome variables and covariates by these categories were evaluated by ANOVA, Kruskall Wallis test or chi-square tests. HR, HRV, amylase and cortisol were transformed using the natural logarithm because of

skewed distribution. We used linear mixed effect models to account for the intraindividual correlation of the repeated measures of the outcomes.

Modeling HR and HRV

Linear mixed effects models were used to estimate associations of sleep with HR and HRV responses to the stress challenge. In these models repeated measures of the outcomes were modeled as a function of stress task period, sleep, and interactions of sleep with task period in order to estimate associations of sleep with stress reactivity and recovery. Stress task period was modeled using three dummy variables representing three periods (with baseline period as reference group): mental stress task, recovery from mental stress, and orthostatic stress task. Up to 7 repeated measures were available for each individual (two at baseline, one during each of the mental stress periods, one during each of the recovery periods from mental stress, and one during the orthostatic challenge). Models included a random intercept for each person (to account for correlation between repeated measures over time within a person) and random slope of mental stress task indicator to allow for interindividual variability in reactivity. Robust standard errors were reported. Covariates were entered in the models as main effects and as two-way interaction terms with each of the stress task periods.

HR/HRV responses to stress challenge analyzed in this study were: (1) mental stress reactivity response (HR/HRV at stress task – HR/HRV at baseline); (2) recovery from stress task response (HR/HRV at recovery – HR/HRV at stress task); (3) orthostatic reactivity response (HR/HRV at orthostatic – HR/HRV at baseline). A more positive value of the HR stress reactivity response meant greater HR reactivity. A more negative value of the HRV stress reactivity response meant greater HRV reactivity during the stress challenge task(s). A more negative value of the HR recovery response meant greater HR recovery. A more positive value of the HRV recovery response meant greater HRV recovery from the stress challenge task(s). (See Figure 4-3)

A sequence of models was run with various adjustments. Model 1 was adjusted for continuous age, gender, race/ethnicity and income-wealth. Model 2 included covariates in Model 1 plus sleep efficiency (when sleep duration was the main outcome) or sleep duration (when sleep efficiency or insomnia was the main outcome) and body mass index, smoking, alcohol use, diabetes and medications

(antihypertensive medications, antidepressants, sympatho-mimetic medications and medications for sleep and mood). Model 3 was further adjusted for covariates included in Model 2 plus apnea and Model 4 was adjusted for covariates included in Model 3 plus naps (hr/day).

Modeling amylase and cortisol

To analyze the associations of sleep measures with amylase/cortisol responses to the stress challenge we simultaneously modeled the responses of amylase to the entire stress challenge protocol using piecewise mixed models with 2 knots. This modeling used all 4 salivary samples, and the knots were chosen based on the time when 2nd and 3rd samples were collected (on average 38 minutes and 55 minutes after the start of the stress challenge protocol). The model estimates associations of predictors with change in amylase/cortisol levels over each response over the entire exam period. Models also included individual random intercept and random slopes on the first and third spline of time. All covariates were centered with respect to the grand mean when included in the models and were entered in the models as main effects and also as two-way interaction terms with the 3 spline pieces. Robust standard errors are reported.

Amylase/cortisol responses to stress challenge analyzed in this study were: (1) mental stress reactivity response (2^{nd} sample – 1^{st} sample); (2) orthostatic reactivity response (3^{rd} sample – 1^{st} sample); (3) recovery from stress task response (4^{th} sample – 2^{nd} sample). A large value of the stress reactivity response meant that amylase/cortisol levels rose sharply after the stress challenge task(s). A large value of the recovery response meant that amylase/cortisol levels fell sharply after the stress challenge). (See **Error! Reference source not found.**)

A similar sequence of models was used as above except that for amylase we adjusted for hormone replacement therapy and inhaled steroids, and for cortisol we did not adjust for sympathomimetic medication. For both amylase and cortisol, we did not adjust for naps.

Results

The 527 participants included 285 women and 242 men from three different race/ethnic backgrounds, Hispanic (42%), African-American (31%), and white (27%). Participant characteristics classified according to sleep duration, sleep efficiency and insomnia are shown in Table 4-1 and Supplemental

Table D-1. The unadjusted mean (± S.D.) log-transformed HR (log(beast/min)), HF-HRV (log(sec^2)) and amylase (log(nmol/L) at baseline, and responses to the stress challenge by sleep duration, sleep efficiency and insomnia are shown in Supplemental Table D-2, Supplemental Table D-3, Supplemental Table D-4, and Supplemental Figure D-1, Supplemental Figure D-2 and Supplemental Figure D-3.

We expect that heart rate and amylase/cortisol increases with mental and orthostatic stress and declines during recovery whereas HF-HRV decreases with mental and orthostatic stress and increases during recovery. In our study, in the whole sample, we observed the expected direction during the stress challenge (see **Error! Reference source not found.**).

Heart rate (HR) and HF-HRV responses to the stress challenge

Table 4-2 shows the estimated log transformed heart rate in beats/min and 95% CIs in heart rate at baseline and in response to the stress challenge comparing short (< 6 hours) and longer (≥ 6 hours) sleep duration, low and higher sleep efficiency and with and without insomnia. There was a difference in baseline levels of heart rate between short sleepers and longer sleepers in models 3 and 4 but no association of sleep duration with heart rate reactivity or recovery (Table 4-2, first column). Neither low sleep efficiency nor insomnia was associated with exaggerated heart rate responses to the stress challenge (Table 4-2, second and third column). When we explored 7 hours as a cut point for sleep duration, participants who slept <7 hours/night had greater heart rate reactivity in models 2 and 3 but not in model 4 (Supplemental Table D-5, second column).

Table 4-3 shows the estimates and 95% CIs in HF-HRV at baseline and responses to the stress challenge between short (< 6 hours) and long (≥ 6 hours) sleep duration, sleep efficiency and insomnia. There was no HF-HRV differences at baseline nor at responses to the stress challenge between participants who slept <6 hours/night that those who slept ≥6 hours/night (Table 4-3, first column). Participants with low sleep efficiency had lower levels of HF-HRV at baseline than those with higher sleep efficiency (estimate at fully adjusted model −0.58, 95% CI −1.03 to −0.14) but there was no association between low sleep efficiency and exaggerated HF-HRV responses to the stress challenge (Table 4-3, second column). Study participants who reported insomnia had greater HF-HRV orthostatic reactivity (fully adjusted model −0.22; 95% CI −0.44 to −0.002; P <.05) than those who did not (Table 4-3, third

column). When we explored a cut point of short sleep duration at 7 hours, participants who slept <7 hours/night had significantly lower HF-HRV levels at baseline than those who slept ≥7 hours/night in all models and also those who slept <7 hours/night showed significantly greater HF-HRV recovery from mental stress than those who slept ≥7 hours/night (Supplemental Table D-6, second column).

We evaluated the effect modification of naps for HR/HF-HRV but did not find evidence that naps moderated the association between sleep duration and HR/HF-HRV responses.

Amylase and cortisol responses to the stress challenge test

Table 4-4 shows the estimates and 95% CIs in amylase at baseline and responses to the stress challenge between short (< 6 hours) and long (≥ 6 hours) sleep duration, sleep efficiency and insomnia. There were no differences in baseline levels of salivary amylase between participants who slept < 6 hours than those who slept ≥6 hours/night (Table 4-4 , first column). Participants with low sleep efficiency had higher levels of amylase at baseline than participants with high sleep efficiency (estimate at fully adjusted model 0.45; 95% CI 0.04 - 0.86). However, sleep efficiency was not associated with amylase responses to the stress challenge test (Table 4-4 , second column). Insomnia (Table 4-4, third column) was not associated with amylase responses to the stress challenge test.

Table 4-5 shows the estimates and 95% CIs in cortisol at baseline and responses to the stress challenge between short (< 6 hours) and long (≥ 6 hours) sleep duration, sleep efficiency and insomnia. Participants who reported insomnia symptoms had higher levels of cortisol at baseline than participants who did not (estimate at fully adjusted model 0.23; 95% CI 0.08 - 0.35). However, insomnia was not associated with cortisol responses to the stress challenge test (Table 4-5 , third column).

Discussion

We found no evidence that short sleep duration is associated with greater heart rate reactivity to mental stress challenge compared to longer sleep duration. We found that participants reporting insomnia symptoms had greater HF-HRV orthostatic reactivity than those who did not. We also found no evidence that short sleep duration and/or low sleep efficiency are associated with greater amylase reactivity to mental stress challenge compared to longer sleep duration and/or high sleep efficiency.

Our data provide some support for the hypothesis that difficulties of sleep may potentiate the effect of stress challenge on the cardiovascular indices, in that insomnia was associated with greater orthostatic reactivity. Although it has been reported that differences between seated position and supine position in the ANS are small,[176] we found that insomniacs had greater HF-HRV orthostatic reactivity than non-insomniacs. We also found differences in heart rate and HF-HRV in the groups of short vs. longer sleep duration and low vs. high sleep efficiency, respectively, at baseline.

Sleep loss may potentiate the effect of the challenge test on the cardiovascular indices as has been shown in a few experimental studies. For example, a study with 18 healthy volunteers,[67] between 19 and 36 years old, examined the relationship between sleep deprivation and CV reactivity. The study found increased normalized LF-HRV and decreased normalized HF-HRV at baseline after 12 hours of sleep deprivation during a reaction-time testing and a seated position. Another study, a crossover-controlled experiment, with 20 healthy young volunteers, examined cardiovascular reactivity to a stress challenge test involving a Stroop color task and a speech task. The speech task had exaggerated greater effect on systolic blood pressure (SBP) reactivity when subjects had had a night of total sleep deprivation than when they had had a night of normal sleep.[68] HR did not differ in the two sleep situations for any of the stress tasks. In a third study, mental stress was linked to greater HR reactivity to and slower HR recovery from the stressors among 28 subjects after 24-hours of total sleep deprivation.[109] In a more recent study, 79 healthy young men aged 18-30 years old underwent actigraphy for sleep parameters for a week before a stress protocol that involved three tasks: Stroop color-word interference, a multisource interference task, and a speech preparation and delivery task, all of them followed by a period of recovery. The study found an association of habitual short sleep duration with decreased HF-HRV during the stress tasks. Short sleep duration was also associated with greater HR during recovery, but the association disappeared after adjustment for naps.[87] To our knowledge, this is the only study that has examined these associations in habitual sleep.

In an extended analysis, when we computed short sleep duration as <7 hours, participants had greater HR reactivity to mental stress than the group with longer sleep duration (≥7 hours) in a model that included demographic and behavioral factors, medications and sleep apnea, but this association was

attenuated when we further adjusted for naps. Naps seemed to compensate for short sleep. In our sample, those who slept fewer hours at night had longer daytime sleep. For example, 173 participants who slept <6h napped for a mean of 60 (± 54) min, and 358 participants who slept <7 hours napped for a mean of 48 (± 42) min whereas longer sleepers, either ≥6 hours or ≥7 hours, napped for a mean of 30 (± 30) minutes. The effect that naps have on people seems to depend on the group of age. For example, in a recent study in healthy young volunteers, 30-minute naps after sleep restriction in a laboratory setting restored urinary norepinephrine levels that had been increased after two hours of sleep restriction the night before.[177] In another study among 104 volunteers with a mean age of 20 years, 45- to 60-minute naps facilitated blood pressure recovery from mental stressors.[178] However, in a large cohort of older women, daily napping for total sleep duration of 8 or 9 hours was associated with increased mortality from all causes excluding cancer.[179] In another cohort of octogenarians, disturbed sleep was associated with higher odds of naps independent of total sleep time.[180]

The influence of sleep characteristics on subsequent hormonal responses to stressors has been examined in animals[169] and in restricted population samples. For example, in a study of 31 children[170] aged 10-17 years old, sleep problems within two weeks prior to the stress challenge were evaluated to stress challenge responses. The protocol involved three stressors: speech, mental arithmetic, and mirror tracing tasks. Subjective poor sleep quality (assessed as sleep-wake behavior problems) was associated with attenuated cortisol responses to stress but short sleep duration was not associated with cortisol stress reactivity. In another study, 64 women, aged 37±10 years,[89] were exposed to a battery of six Stroop color-word interference trials, and 7 day-actigraphy previous to the stress challenge. Poor sleep quality (assessed as low sleep efficiency) measured on the previous night predicted attenuated cortisol reactivity, but short sleep duration did not.

Contrary to previous studies, our study examined how chronic habitual short sleep duration and/or poor sleep quality affects subsequent cortisol responses to acute mental stress in an elderly population. We did not observe an association between sleep quantity or quality and cortisol reactivity. We found no associations between short sleep duration and greater amylase reactivity to mental stress, either.

Our study has some limitations. First, we may have missed the effect of short sleep duration or low sleep efficiency on the hormonal responses to a stress challenge protocol because of lack of power. Second, 88% of our study participants were taking at least one prescribed medication suggesting a high prevalence of medical conditions. Although we either excluded or adjusted for almost all medications that are thought to interact with the secretion of cortisol and/or amylase, we may have either over- or underadjusted our models. In the amylase analyses, for example, we had to exclude 106 participants who used beta blockers. Third, many, perhaps most, of our participants had several chronic conditions that could have attenuated the cortisol responses to acute stress in the laboratory. Fourth, because ours is a cross-sectional study, we cannot draw conclusions about cause and effect. Fifth, the short and longer sleepers and the poor and good sleepers did not differ with respect to stress scores evaluated at baseline and after each mental stressor indicating that the subjective impression of stress was similar in both groups.

However, our study also has strengths. First, the MESA Study is an epidemiological study of a population-based sample with heterogeneous background, from which we collected 4 measures of salivary cortisol and amylase and physiological CV markers (HR and HRV) in response to a standardized stress challenge protocol. To our knowledge, ours is the first study of the amylase response to stress and alterations of sleep in a large community-based sample. Second, we have objective measures of sleep, collected under "normal" habitual circumstances at home without imposing sleep restrictions. We assume that we captured actual sleep habits in our population. Third, we count with extended data of potential confounders in the relationship sleep and markers of stress such as apnea.

In summary, we have not found significant evidence suggesting the association of short sleep duration with greater heart rate reactivity to mental stress challenge compared to longer sleep duration. However, some of our data suggest that insomnia is associated with orthostatic reactivity. We also found differences at baseline on the groups of short and longer sleepers with respect to HR, and on the groups of poor and good sleepers with respect to HF-HRV, amylase and cortisol. This finding is important. We all have to deal with mental stressors in our everyday life, and it is useful to know that reduced sleep

duration and quality may potentiate the effect of the stressors on our cardiovascular/neuroendocrine responses.

Table 4-1. Characteristics of participants (n= 527) by sleep duration, sleep efficiency and insomnia symptoms, MESA Study (2010-2012)

	Sleep Duration		**Sleep Efficiency**		**Insomnia Symptoms**	
	≥ 6 hours (n = 354)	< 6 hours (n = 173)	≥ 85% (n = 473)	< 85% (n = 54)	No (n = 334)	Yes (n = 184)
	N(%) or Mean ± SD					
Demographics						
Age (years)	68.5 ± 8.6	67.7 ± 9.2	68.1 ± 8.9	69.4 ± 7.8	68.5 ± 8.4	67.6 ± 8.9
Male	156 (44)	86(50)	208 (44)	34 (63)*	167 (50)	70 (38)*
Race/ethnicity						
White	116 (33)	27 (16)*	132 (28)	11 (20)	99 (30)	41 (22)
Black	85 (24)	78 (45)	145 (31)	18 (33)	102 (31)	58 (32)
Hispanic	153 (43)	68 (39)	196 (41)	25 (46)	133 (40)	85 (46)
Income-Wealth Index	4.5 ± 2.2	3.9 ± 2.2*	4.3 ± 2.2	4.3 ± 2.4	4.5 ± 2.2	4.0 ± 2.2
Lifestyle Characteristics						
BMI	28.9 ± 5.0	30.7 ± 5.8*	29.4 ± 5.2	30.2 ± 6.2	29.6 ± 5.1	29.3 ± 5.8
Current smokers	150 (42)	65 (38)	40 (9)	4 (7)	26 (8)	16 (9)
Alcohol consumption	76 (45)	139 (39)	193 (41)	22 (41)	14 (42)	69 (38)
Hypertension	202 (57)	106 (61)	276 (58)	32 (59)	201 (60)	103 (56)
Diabetes	142 (41)	67 (39)	180 (38)	29 (54)*	128 (39)	78 (42)
Depression CESD >16	48 (14)	36 (21)*	72 (15)	12 (23)	36 (11)	46 (26)*
Steroid[1] use	5 (1)	14 (8)	3 (6)	16 (3)	14 (4)	5 (3)
Antihypertensive use	192 (54)	104 (60)	264 (56)	32 (59)	189 (57)	102 (55)
Hormone therapy use	16 (5)	4 (2)	20 (4)	0	13 (4)	7 (4)
Antidepressant use[2]	44 (12)	15 (9)	54 (11)	5 (9)	36 (11)	23 (13)
Sleep Variables						
Sleep duration (hrs)	----	----	6.5 ± 1.1	5.7 ± 1.1*	6.5 ± 1.1	6.4 ± 1.2
Sleep efficiency <85%	25 (7)	29 (18)*	----	----	29 (9)	24 (13)
Insomnia symptom[3]	116 (33)	68 (40)	160 (34)	24 (45)	----	----
AHI[4] ≥ 15	184 (56)	93 (62)	240 (56)	37 (76)*	175 (58)	98 (57)
Naps[5] (min/day)	32 ± 34	61 ± 51*	41 ± 42	42 ± 46	40 ± 38	45 ± 49

*p value < 0.05 using either chi-square or t-test/ANOVA or Kruskall Wallis test for non-normally distributed continuous variables.

[1] Oral or inhaled.

[2] Medications for sleep and mood (including benzodiazepines).

[3] WHIIRS score ≥ 9 = insomnia.

[4] AHI = all apneas and hypopnea per sleep hour with ≥ 3% or greater desaturation-index.

[5] All average sleep time in naps per day across all days when only naps of ≥15 minutes duration are counted.

Table 4-2. Mean differences in log-transformed heart rate (log (beats/min)) at baseline and mean differences in responses to challenge by sleep duration, sleep efficiency and insomnia symptoms (N = 3450 observations, 527 persons)

	Sleep Duration* **(< 6 hrs vs. ≥ 6 hrs)** Estimate and 95% C.I.			**Sleep Efficiency (low vs. high)** Estimate and 95% C.I.			**Insomnia (Yes vs. No)** Estimate and 95% C.I.		
Baseline									
Model 1	0.0255	-0.0056	0.0566	0.0255	-0.0248	0.0759	0.0122	-0.0174	0.0417
Model 2	0.0289	-0.0021	0.0598	0.0139	-0.0361	0.0639	0.0106	-0.0187	0.0400
Model 3	**0.0360**	**0.0033**	**0.0686**	0.0112	-0.0411	0.0635	0.0046	-0.0265	0.0357
Model 4	**0.0404**	**0.0070**	**0.0739**	0.0096	-0.0427	0.0620	0.0060	-0.0247	0.0367
Reactivity to Mental Stress[1]									
Model 1	0.0075	-0.0030	0.0180	-0.0029	-0.0175	0.0116	0.0000	-0.0096	0.0097
Model 2	0.0079	-0.0022	0.0181	-0.0046	-0.0189	0.0096	-0.0004	-0.0098	0.0090
Model 3	0.0055	-0.0052	0.0161	-0.0080	-0.0219	0.0060	0.0018	-0.0080	0.0117
Model 4	0.0043	-0.0074	0.0160	-0.0077	-0.0216	0.0062	0.0016	-0.0082	0.0115
Recovery from Mental Stress[2]									
Model 1	0.0005	-0.0080	0.0089	0.0038	-0.0071	0.0147	0.0049	-0.0031	0.0129
Model 2	-0.0003	-0.0086	0.0080	0.0033	-0.0074	0.0141	0.0055	-0.0024	0.0133
Model 3	0.0013	-0.0074	0.0100	0.0047	-0.0060	0.0155	0.0039	-0.0043	0.0122
Model 4	0.0033	-0.0062	0.0128	0.0042	-0.0066	0.0151	0.0044	-0.0039	0.0126
Reactivity to Orthostatic Stress[3]									
Model 1	0.0077	-0.0048	0.0202	-0.0061	-0.0214	0.0092	0.0084	-0.0040	0.0208
Model 2	0.0072	-0.0052	0.0195	-0.0090	-0.0251	0.0071	0.0083	-0.0042	0.0207
Model 3	0.0094	-0.0040	0.0227	-0.0111	-0.0284	0.0061	0.0083	-0.0051	0.0216
Model 4	0.0099	-0.0050	0.0248	-0.0111	-0.0284	0.0062	0.0082	-0.0051	0.0216

*Short sleep duration was categorized as <6 hours vs. longer ≥6 hours (reference)

[1] Reactivity to Mental Stress by Sleep Duration = HR at mental stress - HR at baseline
[2] Recovery from Mental Stress by Sleep Duration = HR at recovery - HR at mental stress
[3] Reactivity to Orthostatic Stress by Sleep Duration = HR at orthostatic stress - HR at baseline

Note: A more positive coefficient for the reactivity associated with shorter sleep means a greater increase in HR response to the stressor (greater reactivity); a more negative coefficient for recovery associated with shorter sleep means a greater HR recovery from the stressor (**Error! Reference source not found.**, Supplemental Table D-2 nd Supplemental Figure D-1)

Model 1 = age, gender, race/ethnicity, income-wealth index.
Model 2 = model 1 plus body mass index, smoking, alcohol consumption, medications (antihypertensive, antidepressants, sympatho-mimetic medications and medication for sleep and mood), diabetes and sleep efficiency.
Model 3 = model 2 plus apnea.
Model 4 = model 3 plus naps (hr/day)

Table 4-3. Mean differences in log-transformed HF-HRV (log (msec^2)) at baseline and mean differences in responses to challenge by sleep duration, sleep efficiency and insomnia symptoms (N = 3450 observations, 527 persons)

	Sleep Duration* (< 6 hrs vs. ≥ 6 hrs) Estimate and 95% C.I.			**Sleep Efficiency (low vs. high)** Estimate and 95% C.I.			**Insomnia (Yes vs. No)** Estimate and 95% C.I.		
Baseline									
Model 1	0.05	–0.23,	0.32	**–0.52**	**–1.01,**	**–0.04**	–0.03	–0.29,	0.23
Model 2	0.06	–0.20,	0.33	–0.48	–0.96,	0.00	–0.03	–0.29,	0.22
Model 3	0.00	–0.28,	0.28	–**0.60**	–**1.04,**	–**0.15**	–0.01	–0.27,	0.25
Model 4	–0.01	–0.31,	0.28	–**0.58**	–**1.03,**	–**0.14**	–0.02	–0.27,	0.23
Reactivity to Mental Stress[1]									
Model 1	–0.08	–0.23,	0.07	–0.05	–0.27,	0.17	–0.14	–0.29,	0.01
Model 2	–0.09	–0.24,	0.06	–0.01	–0.24,	0.21	–0.12	–0.27,	0.03
Model 3	–0.05	–0.21,	0.11	0.12	–0.09,	0.33	–0.13	–0.30,	0.03
Model 4	–0.09	–0.26,	0.09	0.13	–0.08,	0.34	–0.15	–0.31,	0.02
Recovery from Mental Stress[2]									
Model 1	0.04	–0.09,	0.17	0.05	–0.11,	0.21	0.10	–0.02,	0.23
Model 2	0.05	–0.07,	0.18	0.01	–0.16,	0.18	0.08	–0.04,	0.20
Model 3	0.02	–0.12,	0.15	–0.03	–0.21,	0.15	0.08	–0.05,	0.21
Model 4	0.02	–0.13,	0.17	–0.03	–0.21,	0.14	0.09	–0.04,	0.22
Reactivity to Orthostatic Stress[3]									
Model 1	–0.05	–0.24,	0.14	–0.03	–0.24,	0.17	**–0.20**	**–0.41,**	**–0.001**
Model 2	–0.04	–0.23,	0.14	0.00	–0.22,	0.22	–0.20	–0.40,	0.011
Model 3	–0.10	–0.30,	0.10	0.08	–0.15,	0.31	–0.21	–0.44,	0.010
Model 4	–0.16	–0.37,	0.06	0.10	–0.14,	0.33	**–0.22**	**–0.44,**	**–0.002**

*Short sleep duration was categorized as <6 hours vs. longer ≥6 hours (reference)

[1] Reactivity to Mental Stress by Sleep Duration = HF-HRV at mental stress - HF-HRV at baseline
[2] Recovery from Mental Stress by Sleep Duration = HF-HRV at recovery - HF-HRV at mental stress
[3] Reactivity to Orthostatic Stress by Sleep Duration = HF-HRV at orthostatic stress - HF-HRV at baseline

Note: A more negative coefficient for the reactivity associated with shorter sleep means a greater reduction in HF-HRV response to the stressor, a more positive coefficient for recovery associated with shorter sleep means a greater increase in HF-HRV during recovery. (**Error! Reference source not found.**, Supplemental Table D-3 and REF _Ref430434433 \h * MERGEFORMAT Supplemental Figure D-2)

Model 1 = age, gender, race/ethnicity, income-wealth index.
Model 2 = model 1 plus body mass index, smoking, alcohol consumption, medications (antihypertensive, antidepressants, sympatho-mimetic medications and medication for sleep and mood), diabetes and sleep efficiency.
Model 3 = model 2 plus apnea.
Model 4 = model 3 plus naps (hr/day)

Table 4-4. Mean differences in log transformed amylase ((log (U/mL)) at baseline and mean differences in responses to challenge by sleep duration, sleep efficiency and insomnia symptoms (N=1736 observations, 454 persons)

	Sleep Duration (< 6 hrs vs. ≥ 6 hrs) Estimate and 95% C.I.			**Sleep Efficiency low vs. high** Estimate and 95% C.I.			**Insomnia Symptoms Insomnia vs. non-insomnia** Estimate and 95% C.I.		
Baseline									
Model 1	0.04	-0.23,	0.31	0.32	-0.04,	0.69	-0.08	-0.34,	0.18
Model 2	0.04	-0.25,	0.33	**0.41**	**0.01,**	**0.82**	-0.09	-0.36,	0.18
Model 3	0.02	-0.27,	0.31	**0.43**	**0.02,**	**0.83**	-0.08	-0.36,	0.19
Model 4	-0.01	-0.30,	0.29	**0.45**	**0.04,**	**0.86**	-0.08	-0.35,	0.20
Mental stress reactivity[1]									
Model 1	0.14	-0.04,	0.31	-0.08	-0.31,	0.15	0.03	-0.15,	0.21
Model 2	**0.18**	**0.00,**	**0.37**	-0.20	-0.46,	0.06	0.05	-0.13,	0.23
Model 3	0.16	-0.02,	0.35	-0.22	-0.47,	0.04	0.04	-0.14,	0.21
Model 4	0.14	-0.04,	0.33	-0.21	-0.47,	0.04	0.03	-0.15,	0.21
Orthostatic stress reactivity[2]									
Model 1	0.04	-0.17,	0.24	-0.28	-0.63,	0.07	0.11	-0.10,	0.33
Model 2	0.10	-0.12,	0.32	-0.29	-0.68,	0.10	0.13	-0.08,	0.34
Model 3	0.10	-0.12,	0.32	-0.31	-0.70,	0.08	0.10	-0.12,	0.32
Model 4	0.06	-0.17,	0.28	-0.32	-0.73,	0.08	0.08	-0.14,	0.30
Total recovery[3]									
Model 1	-0.16	-0.37,	0.04	-0.07	-0.39,	0.26	0.03	-0.16,	0.23
Model 2	-0.10	-0.31,	0.10	0.06	-0.21,	0.34	0.02	-0.18,	0.23
Model 3	-0.08	-0.29,	0.13	0.05	-0.22,	0.33	0.01	-0.20,	0.22
Model 4	-0.10	-0.31,	0.10	0.05	-0.22,	0.32	-0.01	-0.22,	0.20

[1] the difference between amylase values at 2nd sample (mental stress) and amylase value at 1st sample (baseline)
[2] the difference between amylase values at 3rd sample (orthostatic stress) and amylase value at 1st sample (baseline)
[3] the difference between amylase values at 4th sample (at the end of the stress challenge) and amylase value at 2nd sample (mental stress)

Note: A large value of the stress reactivity response meant that amylase levels rose sharply after the stress challenge task(s). A large value of the recovery response meant that amylase levels fell sharply after the stress challenge (**Error! Reference source not found.**, Supplemental Table D-4 and Supplemental Figure D-3)

Model 1= age, gender race/ethnicity, income wealth index.
Model 2 = model 1 plus apnea and sleep efficiency (for sleep duration as exposure) and sleep duration (for sleep efficiency as exposure).
Model 3 = model 2 plus alcohol consumption, smoking, body mass index.
Model 4 = model 3 plus medications (antihypertensive, antidepressants, hormone replacement therapy, sympatho-mimetic medications, inhaled steroids and medication for sleep and mood) and diabetes

Table 4-5. Mean differences in log transformed cortisol ((log (nmol/L)) at baseline and mean differences in responses to challenge by sleep duration, sleep efficiency and insomnia symptoms (N=2085 observations, 532 persons)

	Sleep Duration (< 6 hrs vs. ≥ 6 hrs) Estimate and 95% C.I.			**Sleep Efficiency low vs. high** Estimate and 95% C.I.			**Insomnia Symptoms Insomnia vs. non-insomnia** Estimate and 95% C.I.		
Baseline									
Model 1	0.02	-0.15	0.18	-0.13	-0.36	0.11	**0.21**	**0.08**	**0.35**
Model 2	0.02	-0.17	0.21	-0.14	-0.41	0.12	**0.22**	**0.07**	**0.37**
Model 3	0.03	-0.17	0.22	-0.14	-0.41	0.12	**0.23**	**0.08**	**0.38**
Model 4	0.04	-0.16	0.23	-0.16	-0.43	0.11	**0.23**	**0.08**	**0.38**
Mental stress reactivity[1]									
Model 1	0.00	-0.10	0.10	-0.07	-0.23	0.09	-0.05	-0.14	0.05
Model 2	-0.01	-0.12	0.11	-0.08	-0.25	0.09	-0.03	-0.14	0.07
Model 3	-0.01	-0.12	0.10	-0.08	-0.24	0.09	-0.04	-0.14	0.06
Model 4	-0.02	-0.13	0.10	-0.07	-0.24	0.10	-0.04	-0.14	0.06
Orthostatic stress reactivity[2]									
Model 1	0.04	-0.10	0.18	-0.02	-0.17	0.13	-0.06	-0.21	0.08
Model 2	0.04	-0.12	0.20	-0.04	-0.19	0.12	-0.09	-0.24	0.07
Model 3	0.04	-0.13	0.20	-0.04	-0.20	0.11	-0.10	-0.25	0.06
Model 4	0.02	-0.14	0.19	-0.03	-0.18	0.13	-0.10	-0.26	0.06
Total recovery[3]									
Model 1	0.02	-0.09	0.12	0.11	-0.03	0.25	0.08	-0.03	0.18
Model 2	0.03	-0.09	0.14	0.12	-0.03	0.26	0.06	-0.05	0.17
Model 3	0.02	-0.09	0.13	0.11	-0.03	0.26	0.05	-0.06	0.16
Model 4	0.02	-0.09	0.13	0.12	-0.02	0.26	0.06	-0.06	0.17

[1] the difference between cortisol values at 2nd sample (mental stress) and cortisol value at 1st sample (baseline)
[2] the difference between cortisol values at 3rd sample (orthostatic stress) and cortisol value at 1st sample (baseline)
[3] the difference between cortisol values at 4th sample (at the end of the stress challenge) and cortisol value at 2nd sample (mental stress)

Model 1= age, gender race/ethnicity, income wealth index.
Model 2 = model 1 plus apnea and sleep efficiency (for sleep duration as exposure) and sleep duration (for sleep efficiency as exposure).
Model 3 = model 2 plus alcohol consumption, smoking, body mass index.
Model 4 = model 3 plus medications (antihypertensive, antidepressants, and medication for sleep and mood) and diabetes

Figure 4-1 Flow Chart of Exclusion Criteria for Participants with Cardiovascular Measures

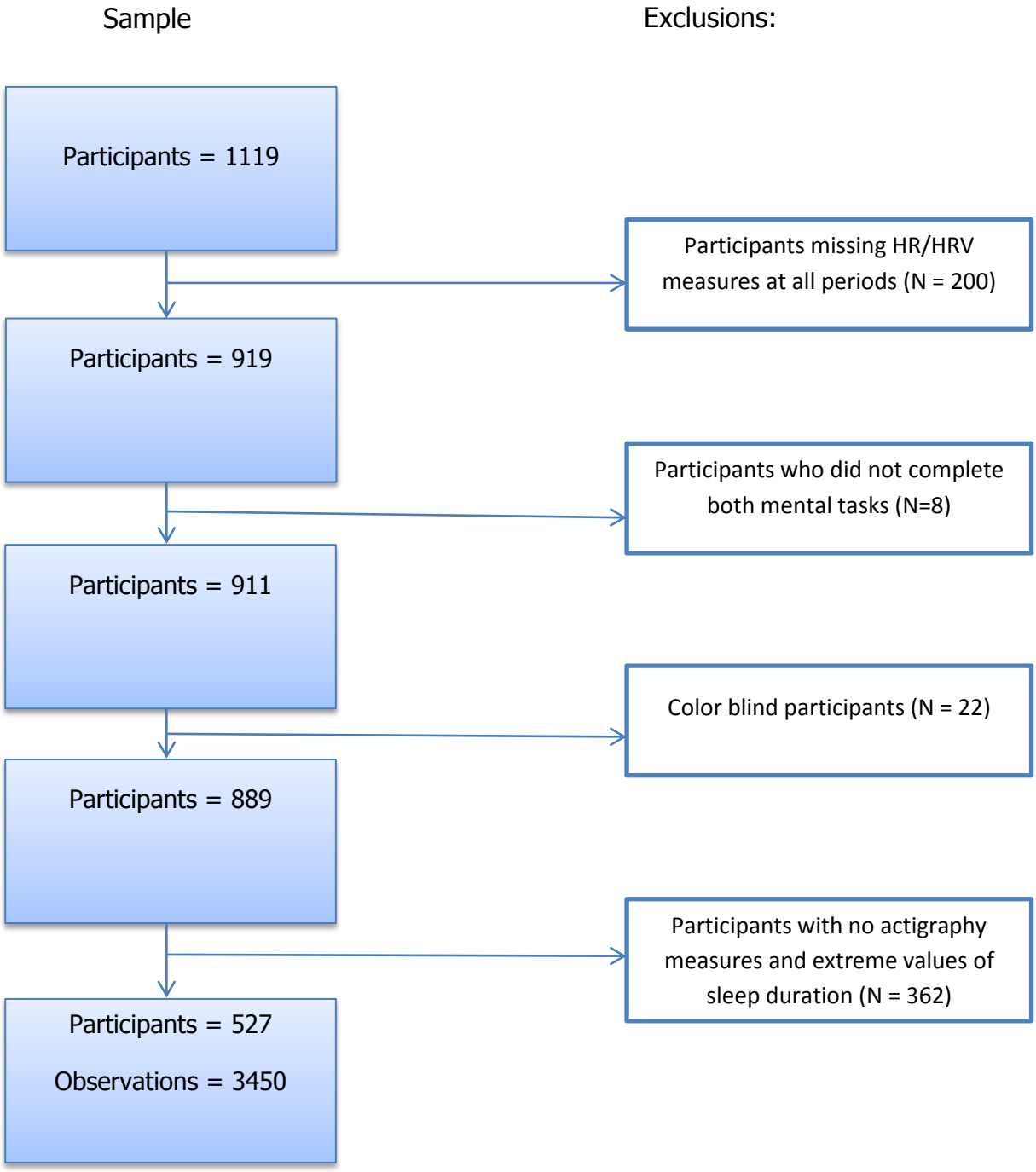


Figure 4-2 Stress challenge protocol: measures of heart rate (HR), HR variability (HRV) and salivary samples (amylase and cortisol)

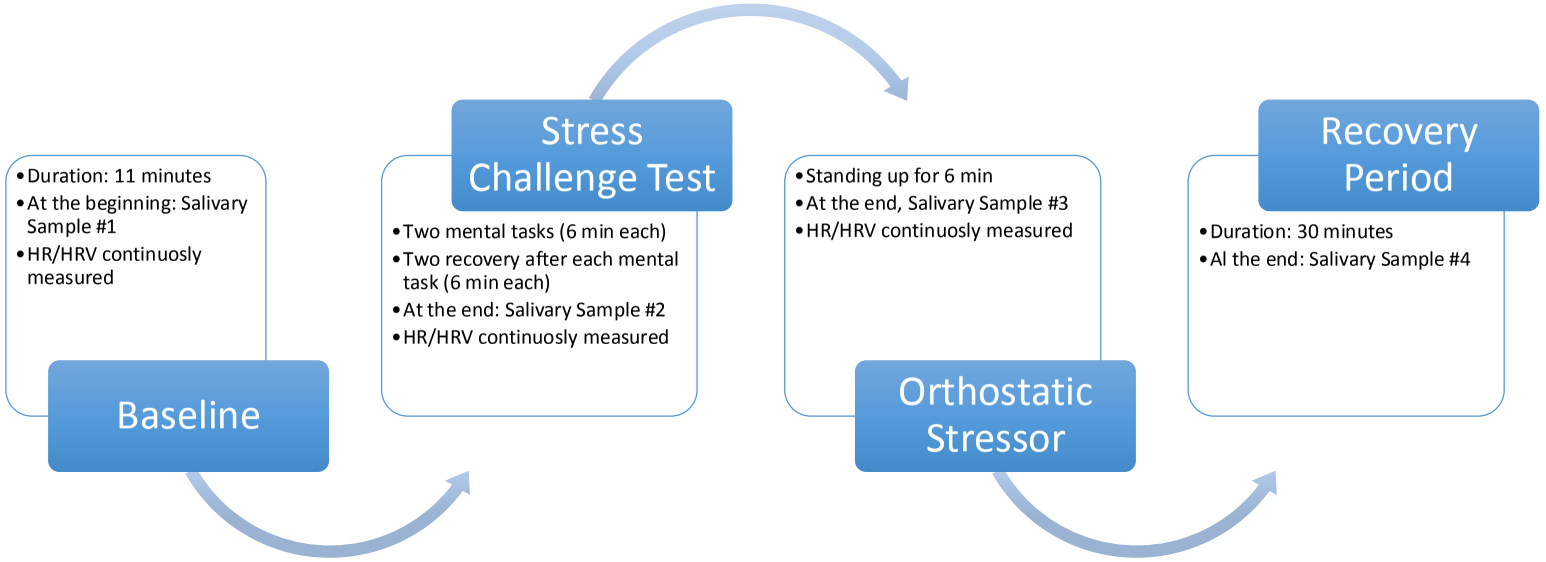
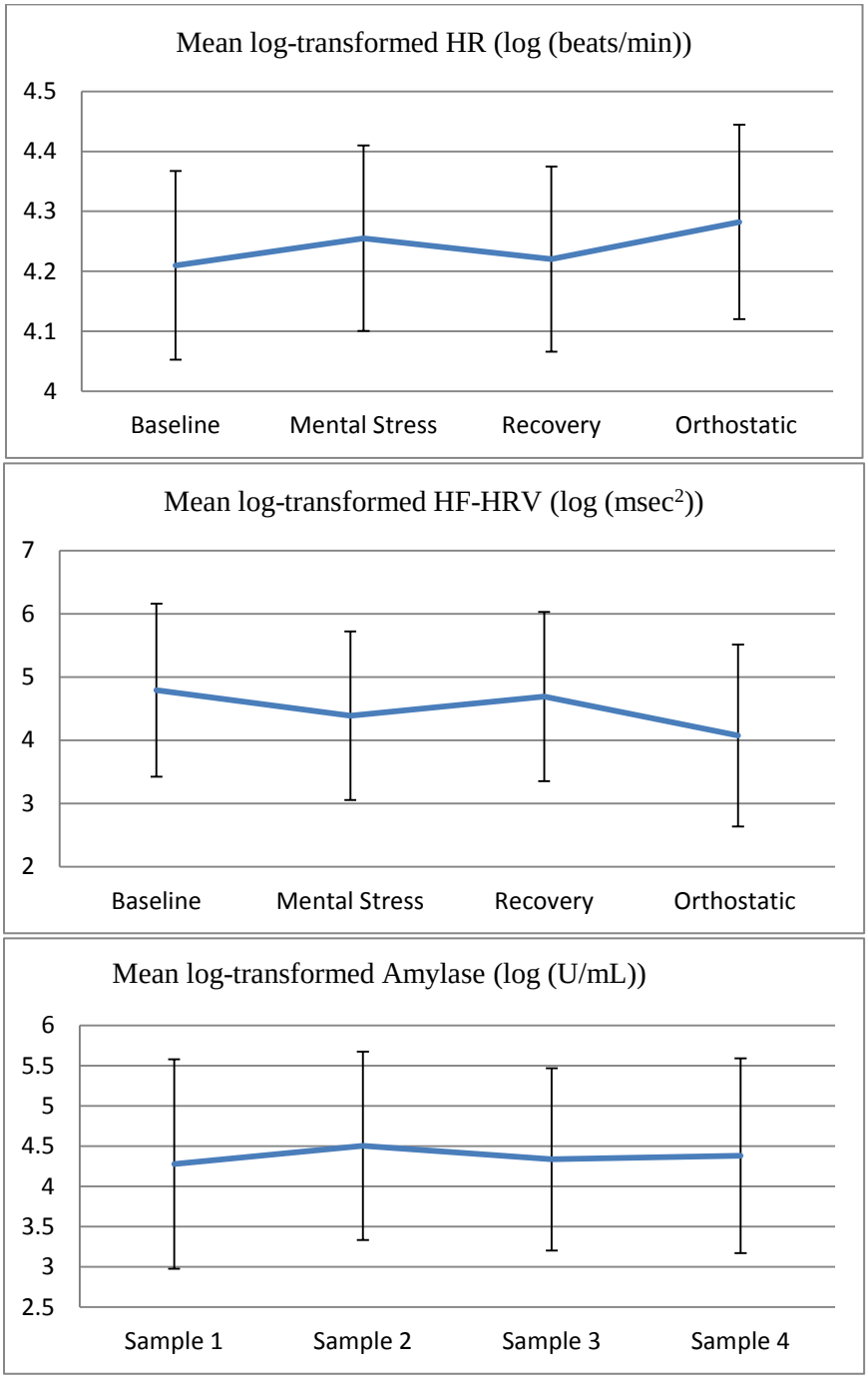


Figure 4-3 Mean Heart Rate (HR), HF-HRV and amylase for the whole population during the MESA Stress protocol



(*Standard deviations are indicated by the error bar*)

For HR/HRV:

Mental Stress = $\frac{Mental\ Stress_1 + Mental\ Stress_2}{2}$

Recovery = $\frac{Recovery\ Period_1 + Recovery\ Period_2}{2}$

Orthostatic = $Orthostatic\ Stress$

For Amylase: *average measure at:*
Sample 1 = at baseline
Sample 2 = at the end of the two stress tasks and respective recovery periods
Sample 3 = at the end of orthostatic stressor
Sample 4 = at 30 minutes of recovery time after end of orthostatic stressor

5. CHAPTER 5. CONCLUSIONS

SLEEP DURATION AND SLEEP EFFICIENCY, THE HYPOTHALAMIC-PITUITARY ADRENOCORTICAL AXIS (HPA) AND THE AUTONOMIC NERVOUS SYSTEM (ANS).

Aims

The overall goal of this dissertation was to study some of the mechanisms that have been proposed to be in the pathway from habitual short sleep duration and sleep disturbances to cardiovascular disease (CVD). We theorized that dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis and overactivation of the sympathetic nervous system (SNS) might be found in this pathway. Short sleep duration and/or poor sleep quality may affect health through specific physiological mechanisms, such as dysfunction of the HPA and/or SNS. When these systems fail to regulate themselves or each other, the body is exposed to adverse events.

The three aims of this dissertation were: 1) to examine the associations of sleep duration and sleep quality with diurnal patterns in salivary cortisol; 2) to examine the association of sleep duration and sleep quality with hormonal measures and cardiovascular markers, in response to a standardized stress challenge protocol; and 3) to test for interaction in the association between short sleep duration and/or poor sleep quality and cortisol with gender, race/ethnicity, and SES.

We hypothesized that adults with short sleep duration and/or poor sleep quality would have altered diurnal cortisol rhythms, in particular flatter cortisol slopes across the day than other adults. We hypothesized that among adults with short sleep duration and/or poor sleep quality, acute and momentary stress would be associated with exaggerated responses of levels of momentary cortisol, amylase and cardiovascular markers during a stress challenge protocol.

Summary of results

In Chapter 2 of this dissertation, a systematic review of the literature identified 19 studies that linked habitual sleep duration/sleep quality with diurnal cortisol measurements in population-based samples of adults. We found only two studies that linked these sleep alterations with responses to a stress challenge protocol in population-based sample of adults. Epidemiological studies have analyzed biological markers of the HPA and SNS in recent years, but few studies have addressed the mechanisms by which

insufficient and poor sleep may be linked to CVD. The 19 papers identified had inconsistent results, perhaps because of non-standardized protocols for sample collection and/or differences in analytical methods. Sample sizes were generally small; only a few studies had sample sizes over 500 participants. Therefore, interpretation of the literature is still problematic.

Chapter 3 of this dissertation assessed the association of diurnal cortisol patterns with habitual short sleep duration and/or poor sleep quality, using either sleep efficiency or symptom of insomnia. We used piecewise mixed linear models, which have been used more recently in the analysis of diurnal cortisol in population-based samples because this analytical method has several advantages over approaches that just summarize repeated observations of the features of the diurnal cortisol such as cortisol awakening response (CAR), diurnal slope, and area under the curve. Mixed models are more efficient than those other approaches because they use all samples, can deal with different numbers of observations per participant, capture the non-linear pattern of the cortisol, and obtain all the diurnal cortisol features from a single model.

We found that sleep duration and efficiency were associated with features of the diurnal cortisol profile in a population-based sample of adults. Persons who slept fewer hours or with lower sleep efficiency had a less pronounced decline in cortisol over the day than persons who slept more hours or had higher sleep efficiency. In addition, study participants with insomnia had a flatter cortisol awakening response (CAR) than those without insomnia, and, in stratified analyses, associations of short sleep duration with a less pronounced decline were stronger in persons who also reported insomnia than in those who did not. The associations of shorter sleep duration with a less pronounced late decline in cortisol over the course of the day were robust to various sensitivity analyses. Other studies have also found alterations in the CAR;[80] our analysis did not show differences, but a trend toward decreased CAR. In general, we observed a flatter pattern in those with short sleep duration than in other subjects, and this pattern was driven by the increase of evening cortisol. This finding was consistent with our hypothesis in which we expected to find alterations of the HPA as a result of sleep loss.

Chapter 4 evaluated the association between habitual short sleep duration and/or poor sleep quality and responses to a standardized stress challenge protocol. We used two types of measures as the

outcomes: hormonal (cortisol and amylase) responses and cardiovascular (heart rate (HR) and HR variability) responses. Cortisol and salivary amylase responses to a stress challenge did not differ by sleep duration group or by sleep efficiency group. The results from this analysis did not support an association between habitual short sleep duration and/or poor sleep quality and responses to a stress challenge protocol. We failed to find evidence that deficient sleep caused an overactivation of the sympathetic nervous system or a withdrawal of the parasympathetic nervous system in response to the stress challenge used in this protocol.

Overall, the results of this dissertation show the need for increasing research on the study of mechanisms by which unsatisfactory sleep causes CVD. Cardiac disease is the leading cause of mortality among men and women in the United States. A large global case-control study, the Interheart,[76] found that more than 90% of the population attributable risk of myocardial infarct is accounted for by nine modifiable risk factors. The risk factors studied were the classic risk factors found by the Framingham study plus several psychosocial factors. However, sleep was not investigated in this large study.

Short sleep duration associated with evening cortisol

In experimental studies, HPA and sleep seem to interact in several ways. The HPA axis affects sleep, but sleep also affects the HPA axis. Sleep onset inhibits cortisol secretion while awakening and sleep offset produce cortisol stimulation.[181] Cortisol has been proposed to be related to health, and it has become important in population surveys. Dysregulation in the regular cortisol diurnal pattern has been associated with diverse pathologies, such as major depression and cancer, and also with CVD mortality in at least one study.[41]

Although the findings are mixed, a consistent pattern that seems to be emerging is the association of adverse outcomes with a flatter decline in cortisol over the day.[182] However, findings for the CAR have been more mixed. For example, a larger CAR and flattened diurnal decline were found to be associated with work-related stress;[146] flatter CAR was associated with burnout;[147] a flatter slope, due to lower morning cortisol, was associated with home-related stress,[145] lower levels at wake-up and less pronounced early decline in cortisol with low-socioeconomic status,[136] and higher evening cortisol with insomnia.[130] A flattened diurnal pattern was also associated with atherosclerosis in the CARDIA study.[144]

Lower CAR was associated with diabetes,[126] and lower levels of awakening cortisol and a less pronounced decline in cortisol were associated with markers of obesity[127] in the MESA Study.

Some investigators[34] have pointed out that sleep loss is a type of stress that can affect health. The HPA axis plays a role in the homeostatic process of the body.[34] Alterations of the activity of both the stress systems, HPA and SNS, have been studied as two of the mechanisms through which sleep loss affects health outcomes in the laboratory and recently in the general population. Biological markers of these systems have been added to epidemiological studies. Cortisol, initially studied in urine and blood and more recently in saliva, is a biological marker of the HPA and is related to behavioral influences and health outcomes.

Responses to stress challenge (acute stress)

In laboratory settings, exposure to stress has been found to lead to cardiovascular and physiological reactivity in population-based epidemiologic studies, but very few studies have examined whether reactivity to stress is different among those who have shorter and/or poorer sleep than in those who have longer sleep duration and/or good sleep quality.

Norepinephrine and epinephrine measures in urine had been the classic markers of the status of the sympathetic nervous system, but they have the disadvantage that they can be measured only in urine or blood; therefore measuring them in large epidemiological studies is a challenge. Most recently, salivary amylase had been thought to be more feasible to use in such studies, but it is note closely correlated with norepinephrine.

We found that sleep duration was associated with evening cortisol; however, sleep duration was not associated with cortisol responses to acute stress. It is possible that the mental tasks were not challenging enough to induce secretion of stress hormones in this population or that participant emotionally withdrew from the challenge at some point during the protocol. It is possible that the response to the challenge depends on "competing stressors," so that the stress in the protocol is not as great at the stress that the study participants face in their daily lives. Or perhaps we did not detect changes in the hormones because our sample lacked statistical power. We had to exclude a number of participants from our pool for different reasons.

Limitations

Although biological markers are more quantifiable than subjective variables, we still know little about the meaning of many biological markers at the population level. A limitation of collecting biological markers (cortisol) from participants at their homes is that it requires a lot of commitment on the part of the participant. Participants need to understand the importance of adhering to the prescribed schedule. For example, if the first cortisol sample of the day is not collected within a few minutes after awakening, we cannot tell whether we missed the CAR or the CAR did not occur. As a result, we may have underestimated the association between sleep duration and the CAR and been unable to demonstrate the importance of studying the relationship between sleep and features of the diurnal cortisol pattern.

A second limitation of this analysis is that the cortisol and stress challenge responses were not assessed simultaneously with the sleep parameters. Among some of the study participants, up to two years elapsed between the biological sample collection and the sleep measurements. However, studies have shown that both sleep and cortisol parameters are relatively stable, as are the cardiovascular responses to the stress challenge.

A third limitation of this analysis is that we may have failed to adjust for all confounders. However, we adjusted for the variables most commonly mentioned and analyzed in the literature relevant to sleep and cortisol.

Finally, we did not find an alteration of the sympathovagal balance related to sleep. Laboratory studies have found that sleep restriction and sleep deprivation result in alterations of the HPA and SNS.[183] However, few studies have addressed the association of these systems with sleep in a population-based sample. We found some evidence suggesting that the HPA is relevant to sleep alterations and CVD, but we did not find comparable evidence of a role for the SNS. Our power to find an effect might have been greater if we had used a composite of several hormones, including inflammatory markers and metabolic markers. All of those markers have been proposed to be in the pathway, and they all work together. Therefore, joining these markers might have been more informative than analyzing each one separately.

Implications

The findings of this dissertation have some important implications. First, people with bad sleep habits need to be aware that adequate sleep in both duration and quality is important to restore the body and to decrease the burden of stress imposed on us over the course of the day.

Evaluating the specific mechanisms by which sleep leads to CVD is essential. If sleep were recognized as a CVD risk factor, the mortality rates for CVD might decline further. Lack of sleep is a type of stressor,[34] and if persistent and chronic it may deregulate normal homeostasis and raise the allostatic load.[184]

We also investigated potential effect modification by gender, age (54 to <68, 68 to < 75 and 75 years old or more) and race/ethnicity (whites, African Americans and Hispanics) in the relationship of sleep duration and/or sleep efficiency and cortisol. We did not find effect modification by these variables. In analyses stratified on race/ethnicity, among whites short sleep duration was significantly associated with lower wake up levels in models 2 through 4, and among blacks, short sleep duration was significantly associated with less pronounced late decline cortisol in all models. In addition, in the group of participants <75 years, short sleep duration was significantly associated with less pronounced late decline in cortisol in all models.

Conclusions

In several laboratory studies, even one night of sleep deprivation in young healthy volunteers has been reported to affect the HPA and to affect the sympathetic/parasympathetic balance. Long-term of lack of sleep in population samples remains to be studied. In animals, lack of sleep and repeated stress causes alteration in some brain regions that involve cognition and feelings. It is reasonable to infer that in humans lack of sleep may produce similar damage. In humans, chronic circadian disruption and reduced sleep time are associated with elevated cortisol, increased obesity, increased oxidative stress and alteration in some brain regions among other consequences.[35] Many hormones, including cortisol, and some behaviors, including sleep, have a circadian pattern.

Experimental studies in both animals and humans have showed that disruption of the circadian cycle[185] produces disruption in some vital functions that, if sustained, may lead to adverse health effects. A common example of circadian disruption is shift work,[186] which has been linked to cardiovascular disease, hypertension, and diabetes. Separately, preferences for a nocturnal chronotype[187] or pattern of sleep-wake activities have also been linked to adverse outcomes such as obesity. For some individuals, a chosen a very early or late sleep may conflict with their regular daily schedule, contributing to insomnia and daytime sleepiness. Studies have reported that later chronotype is associated with unhealthy lifestyle and poorer health behaviors, such as overeating, smoking, alcohol, and lack of physical activity.

Although numerous studies have found a link between sleep loss and CVD, much more research in large population samples is needed to identify the specific mechanisms that account for that link.

1. Hammond EC. Some Preliminary Findings on Physical Complaints from a Prospective Study of 1,064,004 Men and Women. *American journal of public health and the nation's health.* 1964;54:11-23.
2. Kripke DF, Simons RN, Garfinkel L, Hammond EC. Short and long sleep and sleeping pills. Is increased mortality associated? *Archives of general psychiatry.* 1979;36(1):103-116.
3. Heslop P, Smith GD, Metcalfe C, Macleod J, Hart C. Sleep duration and mortality: the effect of short or long sleep duration on cardiovascular and all-cause mortality in working men and women. *Sleep medicine.* 2002;3(4):305-314.
4. McKnight-Eily LR. Unhealthy Sleep-Related Behaviors - 12 States, 2009. *MMWR* 2011;60(8): 233-238.
5. Gallicchio L, Kalesan B. Sleep duration and mortality: a systematic review and meta-analysis. *Journal of sleep research.* 2009;18(2):148-158.
6. Grandner MA, Hale L, Moore M, Patel NP. Mortality associated with short sleep duration: The evidence, the possible mechanisms, and the future. *Sleep Medicine Reviews.* 2010;14(3):191-203.
7. Cappuccio FP, D'Elia L, Strazzullo P, Miller MA. Sleep duration and all-cause mortality: a systematic review and meta-analysis of prospective studies. *Sleep.* 2010;33(5):585-592.
8. Ayas NT, White DP, Manson JE, et al. A prospective study of sleep duration and coronary heart disease in women. *Archives of internal medicine.* 2003;163(2):205-209.
9. Cappuccio FP, Cooper D, D'Elia L, Strazzullo P, Miller MA. Sleep duration predicts cardiovascular outcomes: a systematic review and meta-analysis of prospective studies. *European heart journal.* 2011;32(12):1484-1492.
10. Danielsen YS, Pallesen S, Stormark KM, Nordhus IH, Bjorvatn B. The relationship between school day sleep duration and body mass index in Norwegian children (aged 10-12). *International journal of pediatric obesity : IJPO : an official journal of the International Association for the Study of Obesity.* 2010;5(3):214-220.
11. Lopez-Garcia E, Faubel R, Leon-Munoz L, Zuluaga MC, Banegas JR, Rodriguez-Artalejo F. Sleep duration, general and abdominal obesity, and weight change among the older adult population of Spain. *The American journal of clinical nutrition.* 2008;87(2):310-316.
12. Chaput JP, Despres JP, Bouchard C, Tremblay A. The association between sleep duration and weight gain in adults: a 6-year prospective study from the Quebec Family Study. *Sleep.* 2008;31(4):517-523.
13. Gottlieb DJ, Redline S, Nieto FJ, et al. Association of usual sleep duration with hypertension: the Sleep Heart Health Study. *Sleep.* 2006;29(8):1009-1014.
14. Cappuccio FP, D'Elia L, Strazzullo P, Miller MA. Quantity and quality of sleep and incidence of type 2 diabetes: a systematic review and meta-analysis. *Diabetes care.* 2010;33(2):414-420.
15. Hoevenaar-Blom MP, Spijkerman AM, Kromhout D, van den Berg JF, Verschuren WM. Sleep duration and sleep quality in relation to 12-year cardiovascular disease incidence: the MORGEN study. *Sleep.* 2011;34(11):1487-1492.
16. Foley DJ. An epidemiological perspective on one tale of a two-tailed hypothesis. *Sleep Med Rev.* 2004;8(3):155-157; discussion 175-156.
17. Stamatakis KA, Punjabi NM. Long sleep duration: a risk to health or a marker of risk? *Sleep Med Rev.* 2007;11(5):337-339.
18. Meisinger C, Heier M, Lowel H, Schneider A, Doring A. Sleep duration and sleep complaints and risk of myocardial infarction in middle-aged men and women from the general population: the MONICA/KORA Augsburg cohort study. *Sleep.* 2007;30(9):1121-1127.
19. King CR, Knutson KL, Rathouz PJ, Sidney S, Liu K, Lauderdale DS. Short sleep duration and incident coronary artery calcification. *JAMA.* 2008;300(24):2859-2866.
20. Sands MR, Lauderdale DS, Liu K, et al. Short sleep duration is associated with carotid intima-media thickness among men in the Coronary Artery Risk Development in Young Adults (CARDIA) Study. *Stroke; a journal of cerebral circulation.* 2012;43(11):2858-2864.

21. Faraut B, Touchette E, Gamble H, et al. Short sleep duration and increased risk of hypertension: a primary care medicine investigation. *Journal of hypertension.* 2012;30(7):1354-1363.
22. Gangwisch JE, Heymsfield SB, Boden-Albala B, et al. Short sleep duration as a risk factor for hypertension: analyses of the first National Health and Nutrition Examination Survey. *Hypertension.* 2006;47(5):833-839.
23. Liu R, Zee PC, Chervin RD, et al. Short sleep duration is associated with insulin resistance independent of adiposity in Chinese adult twins. *Sleep medicine.* 2011;12(9):914-919.
24. Knutson KL. Does inadequate sleep play a role in vulnerability to obesity? *American journal of human biology : the official journal of the Human Biology Council.* 2012;24(3):361-371.
25. Leproult R, Van Cauter E. Role of Sleep and Sleep Loss in Hormonal Release and Metabolism. *Endocr Dev.* 2010;17:11-21.
26. Laugsand LE, Vatten LJ, Platou C, Janszky I. Insomnia and the risk of acute myocardial infarction: a population study. *Circulation.* 2011;124(19):2073-2081.
27. Jennings JR, Muldoon MF, Hall M, Buysse DJ, Manuck SB. Self-reported sleep quality is associated with the metabolic syndrome. *Sleep.* 2007;30(2):219-223.
28. Fung MM, Peters K, Redline S, et al. Decreased slow wave sleep increases risk of developing hypertension in elderly men. *Hypertension.* 2011;58(4):596-603.
29. Rao MN, Blackwell T, Redline S, et al. Association between sleep architecture and measures of body composition. *Sleep.* 2009;32(4):483-490.
30. Javaheri S, Storfer-Isser A, Rosen CL, Redline S. Sleep quality and elevated blood pressure in adolescents. *Circulation.* 2008;118(10):1034-1040.
31. Vgontzas AN, Liao D, Pejovic S, Calhoun S, Karataraki M, Bixler EO. Insomnia with objective short sleep duration is associated with type 2 diabetes: A population-based study. *Diabetes care.* 2009;32(11):1980-1985.
32. Fernandez-Mendoza J, Vgontzas AN, Liao D, et al. Insomnia with objective short sleep duration and incident hypertension: the Penn State Cohort. *Hypertension.* 2012;60(4):929-935.
33. Vgontzas AN, Liao D, Bixler EO, Chrousos GP, Vela-Bueno A. Insomnia with objective short sleep duration is associated with a high risk for hypertension. *Sleep.* 2009;32(4):491-497.
34. McEwen BS. Sleep deprivation as a neurobiologic and physiologic stressor: Allostasis and allostatic load. *Metabolism: clinical and experimental.* 2006;55(10 Suppl 2):S20-23.
35. McEwen BS, Karatsoreos IN. Sleep Deprivation and Circadian Disruption: Stress, Allostasis, and Allostatic Load. *Sleep Medicine Clinics.* 2015;10(1):1-10.
36. Black PH, Garbutt LD. Stress, inflammation and cardiovascular disease. *Journal of psychosomatic research.* 2002;52(1):1-23.
37. Palagini L, Bruno RM, Gemignani A, Baglioni C, Ghiadoni L, Riemann D. Sleep Loss and Hypertension: A Systematic Review. *Curr Pharm Design.* 2013;19(13):2409-2419.
38. Axelrod J, Reisine TD. Stress hormones: their interaction and regulation. *Science.* 1984;224(4648):452-459.
39. Tsigos C, Chrousos GP. Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress. *Journal of psychosomatic research.* 2002;53(4):865-871.
40. Vogelzangs N, Beekman AT, Milaneschi Y, Bandinelli S, Ferrucci L, Penninx BW. Urinary cortisol and six-year risk of all-cause and cardiovascular mortality. *The Journal of clinical endocrinology and metabolism.* 2010;95(11):4959-4964.
41. Kumari M, Shipley M, Stafford M, Kivimaki M. Association of diurnal patterns in salivary cortisol with all-cause and cardiovascular mortality: findings from the Whitehall II study. *The Journal of clinical endocrinology and metabolism.* 2011;96(5):1478-1485.
42. Matthews K, Schwartz J, Cohen S, Seeman T. Diurnal cortisol decline is related to coronary calcification: CARDIA study. *Psychosomatic medicine.* 2006;68(5):657-661.
43. Dekker MJ, Koper JW, van Aken MO, et al. Salivary cortisol is related to atherosclerosis of carotid arteries. *The Journal of clinical endocrinology and metabolism.* 2008;93(10):3741-3747.
44. Hamer M, O'Donnell K, Lahiri A, Steptoe A. Salivary cortisol responses to mental stress are associated with coronary artery calcification in healthy men and women. *European heart journal.* 2010;31(4):424-429.

45. Hamer M, Endrighi R, Venuraju SM, Lahiri A, Steptoe A. Cortisol responses to mental stress and the progression of coronary artery calcification in healthy men and women. *Plos One.* 2012;7(2):e31356.
46. Hamer M, Steptoe A. Cortisol responses to mental stress and incident hypertension in healthy men and women. *The Journal of clinical endocrinology and metabolism.* 2012;97(1):E29-34.
47. Leproult R, Copinschi G, Buxton O, Van Cauter E. Sleep loss results in an elevation of cortisol levels the next evening. *Sleep.* 1997;20(10):865-870.
48. Spiegel K, Leproult R, Van Cauter E. Impact of sleep debt on metabolic and endocrine function. *Lancet.* 1999;354(9188):1435-1439.
49. Omisade A, Buxton OM, Rusak B. Impact of acute sleep restriction on cortisol and leptin levels in young women. *Physiology & behavior.* 2010;99(5):651-656.
50. Vgontzas AN, Mastorakos G, Bixler EO, Kales A, Gold PW, Chrousos GP. Sleep deprivation effects on the activity of the hypothalamic-pituitary-adrenal and growth axes: potential clinical implications. *Clinical endocrinology.* 1999;51(2):205-215.
51. Dettenborn L, Rosenloecher F, Kirschbaum C. No effects of repeated forced wakings during three consecutive nights on morning cortisol awakening responses (CAR): a preliminary study. *Psychoneuroendocrinology.* 2007;32(8-10):915-921.
52. Weitzman ED, Fukushima D, Nogeire C, Roffwarg H, Gallagher TF, Hellman L. Twenty-four hour pattern of the episodic secretion of cortisol in normal subjects. *The Journal of clinical endocrinology and metabolism.* 1971;33(1):14-22.
53. Pruessner JC, Wolf OT, Hellhammer DH, et al. Free cortisol levels after awakening: a reliable biological marker for the assessment of adrenocortical activity. *Life sciences.* 1997;61(26):2539-2549.
54. Edwards S, Clow A, Evans P, Hucklebridge F. Exploration of the awakening cortisol response in relation to diurnal cortisol secretory activity. *Life sciences.* 2001;68(18):2093-2103.
55. Kirschbaum C, Pirke KM, Hellhammer DH. The 'Trier Social Stress Test'--a tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology.* 1993;28(1-2):76-81.
56. Frankenhaeuser M, Mellis I, Rissler A, Bjorkvall C, Patkai P. Catecholamine excretion as related to cognitive and emotional reaction patterns. *Psychosomatic medicine.* 1968;30(1):109-124.
57. Turner JR, Hewitt JK, Morgan RK, Sims J, Carroll D, Kelly KA. Graded mental arithmetic as an active psychological challenge. *International journal of psychophysiology : official journal of the International Organization of Psychophysiology.* 1986;3(4):307-309.
58. Mensink GB, Hoffmeister H. The relationship between resting heart rate and all-cause, cardiovascular and cancer mortality. *European heart journal.* 1997;18(9):1404-1410.
59. Sandvik L, Erikssen J, Ellestad M, et al. Heart rate increase and maximal heart rate during exercise as predictors of cardiovascular mortality: a 16-year follow-up study of 1960 healthy men. *Coronary artery disease.* 1995;6(8):667-679.
60. Matthews KA, Katholi CR, McCreath H, et al. Blood Pressure Reactivity to Psychological Stress Predicts Hypertension in the CARDIA Study. *Circulation.* 2004;110(1):74-78.
61. Dodt C, Breckling U, Derad I, Fehm HL, Born J. Plasma Epinephrine and Norepinephrine Concentrations of Healthy Humans Associated With Nighttime Sleep and Morning Arousal. *Hypertension.* 1997;30(1):71-76.
62. Irwin M, Thompson J, Miller C, Gillin JC, Ziegler M. Effects of Sleep and Sleep Deprivation on Catecholamine And Interleukin-2 Levels in Humans: Clinical Implications. *Journal of Clinical Endocrinology & Metabolism.* 1999;84(6):1979-1985.
63. Rasch B, Dodt C, Molle M, Born J. Sleep-stage-specific regulation of plasma catecholamine concentration. *Psychoneuroendocrinology.* 2007;32(8-10):884-891.
64. Roth T. Insomnia: definition, prevalence, etiology, and consequences. *Journal of clinical sleep medicine : JCSM : official publication of the American Academy of Sleep Medicine.* 2007;3(5 Suppl):S7-10.
65. Bonnet MH, Arand DL. Heart rate variability in insomniacs and matched normal sleepers. *Psychosomatic medicine.* 1998;60(5):610-615.

66. Bonnet MH, Arand DL. Hyperarousal and insomnia: state of the science. *Sleep Med Rev.* 2010;14(1):9-15.
67. Zhong X, Hilton HJ, Gates GJ, et al. Increased sympathetic and decreased parasympathetic cardiovascular modulation in normal humans with acute sleep deprivation. *Journal of Applied Physiology.* 2005;98(6):2024-2032.
68. Franzen PL, Gianaros PJ, Marsland AL, et al. Cardiovascular Reactivity to Acute Psychological Stress Following Sleep Deprivation. *Psychosomatic medicine.* 2011;73(8):679-682.
69. Kato M, Phillips BG, Sigurdsson G, Narkiewicz K, Pesek CA, Somers VK. Effects of sleep deprivation on neural circulatory control. *Hypertension.* 2000;35(5):1173-1175.
70. Grassi G, Esler M. How to assess sympathetic activity in humans. *Journal of hypertension.* 1999;17(6):719-734.
71. Rohleder N, Nater UM, Wolf JM, Ehlert U, Kirschbaum C. Psychosocial Stress-Induced Activation of Salivary Alpha-Amylase: An Indicator of Sympathetic Activity? *Annals of the New York Academy of Sciences.* 2004;1032(1):258-263.
72. Anderson LC, Garrett JR, Johnson DA, Kauffman DL, Keller PJ, Thulin A. Influence of circulating catecholamines on protein secretion into rat parotid saliva during parasympathetic stimulation. *The Journal of physiology.* 1984;352:163-171.
73. Chatterton RT, Jr., Vogelsong KM, Lu YC, Ellman AB, Hudgens GA. Salivary alpha-amylase as a measure of endogenous adrenergic activity. *Clinical physiology.* 1996;16(4):433-448.
74. Bosch JA, De Geus EJC, Veerman ECI, Hoogstraten J, Amerongen AVN. Innate secretory immunity in response to laboratory stressors that evoke distinct patterns of cardiac autonomic activity. *Psychosomatic medicine.* 2003;65(2):245-258.
75. Nater UM, La Marca R, Florin L, et al. Stress-induced changes in human salivary alpha-amylase activity -- associations with adrenergic activity. *Psychoneuroendocrinology.* 2006;31(1):49-58.
76. Yusuf S, Hawken S, Ounpuu S, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. *Lancet.* 2004;364(9438):937-952.
77. Baldwin CM, Ervin A, Mays MZ, et al. Sleep Disturbances, Quality of Life, and Ethnicity: The Sleep Heart Health Study. *Journal of Clinical Sleep Medicine.* 2010;6(2):176-183.
78. Chen X, Wang R, Zee P, et al. Racial/Ethnic Differences in Sleep Disturbances: The Multi-Ethnic Study of Atherosclerosis (MESA). *Sleep.* 2015;38(6):877-888.
79. Rao MN, Blackwell T, Redline S, et al. Association between Sleep Duration and 24-Hour Urine Free Cortisol in the MrOS Sleep Study. *Plos One.* 2013;8(9).
80. Kumari M, Badrick E, Ferrie J, Perski A, Marmot M, Chandola T. Self-reported sleep duration and sleep disturbance are independently associated with cortisol secretion in the Whitehall II study. *The Journal of clinical endocrinology and metabolism.* 2009;94(12):4801-4809.
81. Hansen AM, Thomsen JF, Kaergaard A, et al. Salivary cortisol and sleep problems among civil servants. *Psychoneuroendocrinology.* 2012;37(7):1086-1095.
82. Auger RR, Varghese R, Silber MH, Slocumb NL. Total sleep time obtained from actigraphy versus sleep logs in an academic sleep center and impact on further sleep testing. *Nature and science of sleep.* 2013;5:125-131.
83. Lauderdale DS, Knutson KL, Yan LL, Liu K, Rathouz PJ. Self-reported and measured sleep duration: how similar are they? *Epidemiology.* 2008;19(6):838-845.
84. Matthews KA, Woodall KL, Allen MT. Cardiovascular reactivity to stress predicts future blood pressure status. *Hypertension.* 1993;22(4):479-485.
85. Kamarck TW, Jennings JR, Pogue-Geile M, Manuck SB. A multidimensional measurement model for cardiovascular reactivity: stability and cross-validation in two adult samples. *Health psychology : official journal of the Division of Health Psychology, American Psychological Association.* 1994;13(6):471-478.
86. Berntson GG, Cacioppo JT, Binkley PF, Uchino BN, Quigley KS, Fieldstone A. Autonomic cardiac control. III. Psychological stress and cardiac response in autonomic space as revealed by pharmacological blockades. *Psychophysiology.* 1994;31(6):599-608.

87. Mezick EJ, Matthews KA, Hall MH, Richard Jennings J, Kamarck TW. Sleep duration and cardiovascular responses to stress in undergraduate men. *Psychophysiology.* 2014;51(1):88-96.
88. Robillard R, Lanfranchi PA, Prince F, Filipini D, Carrier J. Sleep deprivation increases blood pressure in healthy normotensive elderly and attenuates the blood pressure response to orthostatic challenge. *Sleep.* 2011;34(3):335-339.
89. Wright CE, Valdimarsdottir HB, Erblich J, Bovbjerg DH. Poor sleep the night before an experimental stress task is associated with reduced cortisol reactivity in healthy women. *Biological psychology.* 2007;74(3):319-327.
90. Rueggeberg R, Wrosch C, Miller GE. Sleep duration buffers diurnal cortisol increases in older adulthood. *Psychoneuroendocrinology.* 2012;37(7):1029-1038.
91. Backhaus J, Junghanns K, Hohagen F. Sleep disturbances are correlated with decreased morning awakening salivary cortisol. *Psychoneuroendocrinology.* 2004;29(9):1184-1191.
92. Seelig E, Keller U, Klarhofer M, et al. Neuroendocrine regulation and metabolism of glucose and lipids in primary chronic insomnia: a prospective case-control study. *Plos One.* 2013;8(4):e61780.
93. Eek F, Karlson B, Garde AH, Hansen AM, Orbaek P. Cortisol, sleep, and recovery - Some gender differences but no straight associations. *Psychoneuroendocrinology.* 2012;37(1):56-64.
94. Luik AI, Direk N, Zuurbier LA, Hofman A, Van Someren EJ, Tiemeier H. Sleep and 24-h activity rhythms in relation to cortisol change after a very low-dose of dexamethasone. *Psychoneuroendocrinology.* 2015;53:207-216.
95. Zhang J, Lam SP, Li SX, et al. A community-based study on the association between insomnia and hypothalamic-pituitary-adrenal axis: sex and pubertal influences. *The Journal of clinical endocrinology and metabolism.* 2014;99(6):2277-2287.
96. Garde AH, Albertsen K, Persson R, Hansen ÖM, Rugulies R. Bi-Directional Associations Between Psychological Arousal, Cortisol, and Sleep. *Behavioral sleep medicine.* 2011;10(1):28-40.
97. Lasikiewicz N, Hendrickx H, Talbot D, Dye L. Exploration of basal diurnal salivary cortisol profiles in middle-aged adults: associations with sleep quality and metabolic parameters. *Psychoneuroendocrinology.* 2008;33(2):143-151.
98. Hanson EK, Maas CJ, Meijman TF, Godaert GL. Cortisol secretion throughout the day, perceptions of the work environment, and negative affect. *Annals of behavioral medicine : a publication of the Society of Behavioral Medicine.* 2000;22(4):316-324.
99. Vargas I, Lopez-Duran N. Dissecting the impact of sleep and stress on the cortisol awakening response in young adults. *Psychoneuroendocrinology.* 2014;40:10-16.
100. Minkel J, Moreta M, Muto J, et al. Sleep deprivation potentiates HPA axis stress reactivity in healthy adults. *Health psychology : official journal of the Division of Health Psychology, American Psychological Association.* 2014;33(11):1430-1434.
101. O'Leary ED, Siobhan H, Hughes BM, James Je. Salivary-alpha-Amylase Reactivity to Laboratory Social Stress With and Without Acute Sleep Restriction. *Journal of Psychophysiology.* 2015;29(2):55-63.
102. Hill Golden S, Sanchez BN, Desantis AS, et al. Salivary cortisol protocol adherence and reliability by socio-demographic features: the Multi-Ethnic Study of Atherosclerosis. *Psychoneuroendocrinology.* 2014;43:30-40.
103. Adam EK, Kumari M. Assessing salivary cortisol in large-scale, epidemiological research. *Psychoneuroendocrinology.* 2009;34(10):1423-1436.
104. Adam EK, Hawkley LC, Kudielka BM, Cacioppo JT. Day-to-day dynamics of experience--cortisol associations in a population-based sample of older adults. *Proceedings of the National Academy of Sciences of the United States of America.* 2006;103(45):17058-17063.
105. Vreeburg SA, Hoogendijk WJ, van Pelt J, et al. Major depressive disorder and hypothalamic-pituitary-adrenal axis activity: results from a large cohort study. *Archives of general psychiatry.* 2009;66(6):617-626.
106. Dienes KA, Hazel NA, Hammen CL. Cortisol secretion in depressed, and at-risk adults. *Psychoneuroendocrinology.* 2013;38(6):927-940.
107. Sephton SE, Sapolsky RM, Kraemer HC, Spiegel D. Diurnal cortisol rhythm as a predictor of breast cancer survival. *Journal of the National Cancer Institute.* 2000;92(12):994-1000.

108. Marmot M, Brunner E. Cohort Profile: The Whitehall II study. *International Journal of Epidemiology.* 2005;34(2):251-256.
109. Yang H, Durocher JJ, Larson RA, DellaValla JP, Carter JR. Total sleep deprivation alters cardiovascular reactivity to acute stressors in humans. *Journal of Applied Physiology.* 2012;113(6):903-908.
110. Ekstedt M, Akerstedt T, Soderstrom M. Microarousals during sleep are associated with increased levels of lipids, cortisol, and blood pressure. *Psychosomatic medicine.* 2004;66(6):925-931.
111. Prinz PN, Bailey SL, Woods DL. Sleep impairments in healthy seniors: roles of stress, cortisol, and interleukin-1 beta. *Chronobiology international.* 2000;17(3):391-404.
112. Prinz P, Bailey S, Moe K, Wilkinson C, Scanlan J. Urinary free cortisol and sleep under baseline and stressed conditions in healthy senior women: effects of estrogen replacement therapy. *Journal of sleep research.* 2001;10(1):19-26.
113. Rydstedt LW, Devereux JJ. Psychosocial job strain and sleep quality interaction leading to insufficient recovery. *International journal of environmental research and public health.* 2013;10(11):5863-5873.
114. Shaver JL, Johnston SK, Lentz MJ, Landis CA. Stress exposure, psychological distress, and physiological stress activation in midlife women with insomnia. *Psychosomatic medicine.* 2002;64(5):793-802.
115. Zhang J, Ma RC, Kong AP, et al. Relationship of sleep quantity and quality with 24-hour urinary catecholamines and salivary awakening cortisol in healthy middle-aged adults. *Sleep.* 2011;34(2):225-233.
116. Ayas NT, White DP, Al-Delaimy WK. A prospective study of self-reported sleep duration and incident diabetes in women. *Diabetes care.* 2003;26(2):380-384.
117. Chaput J-P, Despres J-P, Bouchard C, Astrup A, Tremblay A. Sleep duration as a risk factor for the development of type 2 diabetes or impaired glucose tolerance: Analyses of the Quebec Family Study. *Sleep medicine.* 2009;10(8):919-924.
118. Patel SR, Blackwell T, Redline S, et al. The association between sleep duration and obesity in older adults. *International journal of obesity.* 2008;32(12):1825-1834.
119. Ferrie JE, Shipley MJ, Cappuccio FP, et al. A prospective study of change in sleep duration: associations with mortality in the Whitehall II cohort. *Sleep.* 2007;30(12):1659-1666.
120. Li Y, Zhang X, Winkelman JW, et al. Association between insomnia symptoms and mortality: a prospective study of U.S. men. *Circulation.* 2014;129(7):737-746.
121. Mallon L, Broman JE, Hetta J. Sleep complaints predict coronary artery disease mortality in males: a 12-year follow-up study of a middle-aged Swedish population. *Journal of internal medicine.* 2002;251(3):207-216.
122. Kirschbaum C, Hellhammer DH. Salivary cortisol in psychobiological research: an overview. *Neuropsychobiology.* 1989;22(3):150-169.
123. Stawski RS, Almeida DM, Lachman ME, Tun PA, Rosnick CB, Seeman T. Associations between cognitive function and naturally occurring daily cortisol during middle adulthood: timing is everything. *The journals of gerontology. Series B, Psychological sciences and social sciences.* 2011;66 Suppl 1:i71-81.
124. Huber TJ, Issa K, Schik G, Wolf OT. The cortisol awakening response is blunted in psychotherapy inpatients suffering from depression. *Psychoneuroendocrinology.* 2006;31(7):900-904.
125. Bhattacharyya MR, Molloy GJ, Steptoe A. Depression is associated with flatter cortisol rhythms in patients with coronary artery disease. *Journal of psychosomatic research.* 2008;65(2):107-113.
126. Champaneri S, Xu X, Carnethon MR, et al. Diurnal salivary cortisol and urinary catecholamines are associated with diabetes mellitus: the Multi-Ethnic Study of Atherosclerosis. *Metabolism: clinical and experimental.* 2012;61(7):986-995.
127. Champaneri S, Xu X, Carnethon MR, et al. Diurnal salivary cortisol is associated with body mass index and waist circumference: the Multiethnic Study of Atherosclerosis. *Obesity.* 2013;21(1):E56-63.

128. Vgontzas AN, Fernandez-Mendoza J, Liao D, Bixler EO. Insomnia with objective short sleep duration: the most biologically severe phenotype of the disorder. *Sleep Med Rev.* 2013;17(4):241-254.
129. Vgontzas AN, Liao D, Pejovic S, et al. Insomnia with short sleep duration and mortality: the Penn State cohort. *Sleep.* 2010;33(9):1159-1164.
130. Vgontzas AN, Bixler EO, Lin HM, et al. Chronic insomnia is associated with nyctohemeral activation of the hypothalamic-pituitary-adrenal axis: clinical implications. *The Journal of clinical endocrinology and metabolism.* 2001;86(8):3787-3794.
131. Rodenbeck A, Huether G, Rüther E, Hajak G. Interactions between evening and nocturnal cortisol secretion and sleep parameters in patients with severe chronic primary insomnia. *Neuroscience letters.* 2002;324(2):159-163.
132. Riemann D, Klein T, Rodenbeck A, et al. Nocturnal cortisol and melatonin secretion in primary insomnia. *Psychiatry research.* 2002;113(1–2):17-27.
133. Bild DE, Bluemke DA, Burke GL, et al. Multi-Ethnic Study of Atherosclerosis: objectives and design. *Am J Epidemiol.* 2002;156(9):871-881.
134. Oakley NR. Validation with polysomnography of the Sleepwatch sleep/wake scoring algorithm used by the Actiwatch activity monitoring system. Bend: Mini Mitter, Cambridge Neurotechnology. 1997.
135. Levine DW, Dailey ME, Rockhill B, Tipping D, Naughton MJ, Shumaker SA. Validation of the Women's Health Initiative Insomnia Rating Scale in a multicenter controlled clinical trial. *Psychosomatic medicine.* 2005;67(1):98-104.
136. Hajat A, Diez-Roux A, Franklin TG, et al. Socioeconomic and race/ethnic differences in daily salivary cortisol profiles: the multi-ethnic study of atherosclerosis. *Psychoneuroendocrinology.* 2010;35(6):932-943.
137. Barefoot JC, Dodge KA, Peterson BL, Dahlstrom WG, Williams RB, Jr. The Cook-Medley hostility scale: item content and ability to predict survival. *Psychosomatic medicine.* 1989;51(1):46-57.
138. Radloff LS. The CES-D Scale: A Self-Report Depression Scale for Research in the General Population. *Applied Psychological Measurement* 1977;1:385-401.
139. Golden SH, Lee HB, Schreiner PJ, et al. Depression and type 2 diabetes mellitus: the multiethnic study of atherosclerosis. *Psychosomatic medicine.* 2007;69(6):529-536.
140. Ranjit N, Diez-Roux AV, Sanchez B, et al. Association of salivary cortisol circadian pattern with cynical hostility: multi-ethnic study of atherosclerosis. *Psychosomatic medicine.* 2009;71(7):748-755.
141. Voderholzer U, Piosczyk H, Holz J, et al. The impact of increasing sleep restriction on cortisol and daytime sleepiness in adolescents. *Neuroscience letters.* 2012;507(2):161-166.
142. Enders CK, Tofighi D. Centering predictor variables in cross-sectional multilevel models: a new look at an old issue. *Psychological methods.* 2007;12(2):121-138.
143. Hajat A, Diez-Roux AV, Sanchez BN, et al. Examining the association between salivary cortisol levels and subclinical measures of atherosclerosis: the Multi-Ethnic Study of Atherosclerosis. *Psychoneuroendocrinology.* 2013;38(7):1036-1046.
144. Matthews KA, Zhu S, Tucker DC, Whooley MA. Blood Pressure Reactivity to Psychological Stress and Coronary Calcification in the Coronary Artery Risk Development in Young Adults Study. *Hypertension.* 2006;47(3):391-395.
145. Adam EK, Gunnar MR. Relationship functioning and home and work demands predict individual differences in diurnal cortisol patterns in women. *Psychoneuroendocrinology.* 2001;26(2):189-208.
146. Karlson B, Eek F, Hansen ÅM, Garde AH, Ørbæk P. Cortisol variability and self-reports in the measurement of work-related stress. *Stress & Health: Journal of the International Society for the Investigation of Stress.* 2011;27(2):e11-e24.
147. Pruessner JC, Hellhammer DH, Kirschbaum C. Burnout, perceived stress, and cortisol responses to awakening. *Psychosomatic medicine.* 1999;61(2):197-204.
148. Ice GH, Katz-Stein A, Himes J, Kane RL. Diurnal cycles of salivary cortisol in older adults. *Psychoneuroendocrinology.* 2004;29(3):355-370.

149. Luecken L, Gallo L. *Handbook of Physiological Research Methods in Health Psychology.* California, USA: Sage Publications, Inc; 2008.
150. Akerstedt T. Psychosocial stress and impaired sleep. *Scand J Work Environ Health.* 2006;32(6):493-501.
151. Knutson KL, Rathouz PJ, Yan LL, Liu K, Lauderdale DS. Intra-individual daily and yearly variability in actigraphically recorded sleep measures: the CARDIA study. *Sleep.* 2007;30(6):793-796.
152. Wang X SB, Golden SH, Shrager S, Kirschbaum C, Karlamangla AS, Seeman TE, Diez Roux AV. Stability and predictors of change in salivary cortisol measures over six years: MESA. *Psychoneuroendocrinology.* 2014;49:310-320.
153. Redline S, Foody J. Sleep Disturbances: Time to Join the Top 10 Potentially Modifiable Cardiovascular Risk Factors? *Circulation.* 2011;124(19):2049-2051.
154. Carroll D, Smith GD, Shipley MJ, Steptoe A, Brunner EJ, Marmot MG. Blood pressure reactions to acute psychological stress and future blood pressure status: a 10-year follow-up of men in the Whitehall II study. *Psychosomatic medicine.* 2001;63(5):737-743.
155. Jennings JR, Kamarck TW, Everson-Rose SA, Kaplan GA, Manuck SB, Salonen JT. Exaggerated Blood Pressure Responses During Mental Stress Are Prospectively Related to Enhanced Carotid Atherosclerosis in Middle-Aged Finnish Men. *Circulation.* 2004;110(15):2198-2203.
156. Steptoe A, Donald AE, O'Donnell K, Marmot M, Deanfield JE. Delayed blood pressure recovery after psychological stress is associated with carotid intima-media thickness: Whitehall psychobiology study. *Arteriosclerosis, thrombosis, and vascular biology.* 2006;26(11):2547-2551.
157. Reyes del Paso GA, Langewitz W, Mulder LJ, van Roon A, Duschek S. The utility of low frequency heart rate variability as an index of sympathetic cardiac tone: a review with emphasis on a reanalysis of previous studies. *Psychophysiology.* 2013;50(5):477-487.
158. Thayer JF, Sternberg E. Beyond heart rate variability: vagal regulation of allostatic systems. *Annals of the New York Academy of Sciences.* 2006;1088:361-372.
159. Stein P, P.K., Kleiger M, R.E. Insights from the Study of Heart Rate Variability *Annual Review of Medicine.* 1999;50(1):249-261.
160. Somers VK, Dyken ME, Mark AL, Abboud FM. Sympathetic-nerve activity during sleep in normal subjects. *The New England journal of medicine.* 1993;328(5):303-307.
161. Barnett KJ, Cooper NJ. The effects of a poor night sleep on mood, cognitive, autonomic and electrophysiological measures. *Journal of integrative neuroscience.* 2008;7(3):405-420.
162. Sauvet F, Leftheriotis G, Gomez-Merino D, et al. Effect of acute sleep deprivation on vascular function in healthy subjects. *J Appl Physiol (1985).* 2010;108(1):68-75.
163. Tasali E, Leproult R, Ehrmann DA, Van Cauter E. Slow-wave sleep and the risk of type 2 diabetes in humans. *Proceedings of the National Academy of Sciences of the United States of America.* 2008;105(3):1044-1049.
164. Rohleder N, Nater UM. Determinants of salivary alpha-amylase in humans and methodological considerations. *Psychoneuroendocrinology.* 2009;34(4):469-485.
165. Raikkonen K, Matthews KA, Pesonen AK, et al. Poor sleep and altered hypothalamic-pituitary-adrenocortical and sympatho-adrenal-medullary system activity in children. *The Journal of clinical endocrinology and metabolism.* 2010;95(5):2254-2261.
166. Young EA, Abelson J, Lightman SL. Cortisol pulsatility and its role in stress regulation and health. *Frontiers in neuroendocrinology.* 2004;25(2):69-76.
167. Kirschbaum C, Strasburger CJ, Langkrar J. Attenuated cortisol response to psychological stress but not to CRH or ergometry in young habitual smokers. *Pharmacology, biochemistry, and behavior.* 1993;44(3):527-531.
168. Burke HM, Davis MC, Otte C, Mohr DC. Depression and cortisol responses to psychological stress: a meta-analysis. *Psychoneuroendocrinology.* 2005;30(9):846-856.
169. Meerlo P, Koehl M, van der Borght K, Turek FW. Sleep restriction alters the hypothalamic-pituitary-adrenal response to stress. *Journal of neuroendocrinology.* 2002;14(5):397-402.
170. Capaldi Ii VF, Handwerger K, Richardson E, Stroud LR. Associations between sleep and cortisol responses to stress in children and adolescents: a pilot study. *Behavioral sleep medicine.* 2005;3(4):177-192.

171. Berntson GG QK, Lang JF, Boysen ST. An approach to artifact identification: Application to heart period data. *Psychophysiology.* 1990;27:586-598.
172. Dykes FD AP, Baldzer K, Carrigan TA, Kitney RI, Giddens DP. Breath amplitude modulation of heart rate variability in normal full term neonates. *Pediatric Research.* 1986;20:301.
173. DeBoer RW KJ, Strackee J. Comparing spectra of a series of point events, particularly for heart rate variability spectra. IEEE Transactions in Biomedical Engineering. *BME.* 1984;31:384-387.
174. Harris F. On the use of windows for harmonic analysis with the discrete Fourier transform. *Proceedings of the IEEE.* 1978;66:51-83.
175. Valentini M, Parati G. Variables influencing heart rate. *Prog Cardiovasc Dis.* 2009;52(1):11-19.
176. Sloan RP, McCreath H, Tracey KJ, Sidney S, Liu K, Seeman T. RR interval variability is inversely related to inflammatory markers: the CARDIA study. *Molecular medicine.* 2007;13(3-4):178-184.
177. Faraut B, Nakib S, Drogou C, et al. Napping reverses the salivary interleukin-6 and urinary norepinephrine changes induced by sleep restriction. *The Journal of clinical endocrinology and metabolism.* 2015;100(3):E416-426.
178. Brindle RC, Conklin SM. Daytime sleep accelerates cardiovascular recovery after psychological stress. *Int J Behav Med.* 2012;19(1):111-114.
179. Stone KL, Ewing SK, Ancoli-Israel S, et al. Self-reported sleep and nap habits and risk of mortality in a large cohort of older women. *Journal of the American Geriatrics Society.* 2009;57(4):604-611.
180. Goldman SE, Hall M, Boudreau R, et al. Association between nighttime sleep and napping in older adults. *Sleep.* 2008;31(5):733-740.
181. Balbo M, Leproult R, Van Cauter E. Impact of sleep and its disturbances on hypothalamo-pituitary-adrenal axis activity. *International journal of endocrinology.* 2010;2010:759234.
182. Kumari M, Badrick E, Sacker A, Kirschbaum C, Marmot M, Chandola T. Identifying patterns in cortisol secretion in an older population. Findings from the Whitehall II study. *Psychoneuroendocrinology.* 2010;35(7):1091-1099.
183. Spiegel K, Leproult R, L'Hermite-Baleriaux M, Copinschi G, Penev PD, Van Cauter E. Leptin levels are dependent on sleep duration: relationships with sympathovagal balance, carbohydrate regulation, cortisol, and thyrotropin. *The Journal of clinical endocrinology and metabolism.* 2004;89(11):5762-5771.
184. McEwen BS. Protective and damaging effects of stress mediators. *The New England journal of medicine.* 1998;338(3):171-179.
185. Rajaratnam SM, Arendt J. Health in a 24-h society. *Lancet.* 2001;358(9286):999-1005.
186. Monk TH, Folkard S, Wedderburn AI. Maintaining safety and high performance on shiftwork. *Applied ergonomics.* 1996;27(1):17-23.
187. Karatsoreos IN, Bhagat S, Bloss EB, Morrison JH, McEwen BS. Disruption of circadian clocks has ramifications for metabolism, brain, and behavior. *Proceedings of the National Academy of Sciences of the United States of America.* 2011;108(4):1657-1662.

LIST OF SUPPLEMENTAL TABLES

Supplemental Table B-1 Sleep duration and derived cortisol variables of salivary diurnal cortisol used in epidemiological studies 101

Supplemental Table B-2 Sleep quality and indices of salivary diurnal cortisol and covariates used in epidemiological studies 103

Supplemental Table C-1 First sample time (in minutes) since self-reported wake up after deleting exclusions, MESA Study 106

Supplemental Table C-2 Number of samples per subjects (after exclusions), MESA Study 107

Supplemental Table C-3 . Number of samples per day (after exclusions) 108

Supplemental Table C-4 Distribution of salivary sample collection time (hours), MESA. 108

Supplemental Table C-5 Characteristics of cortisol data collection by demographic characteristics from MESA Stress participants 110

Supplemental Table C-6 Characteristics of participants (n = 600) by mean sleep duration in hours (mean (SD)), MESA Study (2010-2012) 111

Supplemental Table C-7 Percent differences (95% confidence intervals) in features of the daily cortisol associated with sleep duration (≥3 hours to <9 hours) and sleep efficiency (%), specified as continuous, MESA Study 113

Supplemental Table C-8 Percent differences (95% confidence intervals) in features of the daily cortisol associated with sleep duration specified as dichotomized and continuous when participants were restricted to sleep duration < 8 hours 114

Supplemental Table C-9 Percent differences (95% confidence intervals) in wake−to−bed slope of salivary cortisol level associated with sleep duration dichotomized at 5 hours (first column) and at 7 hours (second column) for the overall sample and when restricted, MESA 115

Supplemental Table D-1 Characteristics of participants (n= 527) by sleep duration at < 6 hours, between 6 and less than 7 hours and 7 or more hours, MESA 120

Supplemental Table D-2 Mean Value* log-transformed heart rate (log (beats/min)) at baseline and responses to the stress challenge by sleep duration (SD), sleep efficiency (SE) and insomnia 121

Supplemental Table D-3 Mean value* log-transformed high frequency HR variability (log($msec^2$)) at baseline and responses to the stress by sleep duration (SD), sleep efficiency (SE) and insomnia 122

Supplemental Table D-4 Mean values* of amylase (log(U/mL)) at each sample, reactivity and recovery by sleep duration (SD), sleep efficiency (SE) and insomnia. 123

Supplemental Table D-5 Mean differences in log transformed heart rate (log (beats/min)) at baseline and mean differences in responses to challenge by sleep duration, sleep efficiency and insomnia symptoms (N = 3450 observations, 527 persons) 124

Supplemental Table D-6 Mean differences in log transformed HF-HRV (log($msec^2$)) at baseline and mean differences in responses to challenge by sleep duration, sleep efficiency and insomnia symptoms (N = 3450 observations, 527 persons) 125

LIST OF SUPPLEMENTAL FIGURES

Supplemental Figure A-1 Probable mechanisms involved in the association of short/poor sleep and CVD outcomes 100

Supplemental Figure C-1 Chart of flow of exclusion of participants, days or salivary samples 116

Supplemental Figure C-2 LOESS Plot log transformed cortisol by sleep duration 117

Supplemental Figure C-3 LOESS plot log-transformed cortisol by sleep efficiency and insomnia 118

Supplemental Figure C-4 LOESS plot log-transformed cortisol by gender, race/ethnicity and age 119

Supplemental Figure D-1 Mean log-transformed heart rate (HR) (log beats/min) during the stress challenge protocol, Mesa Stress 126

Supplemental Figure D-2 Mean log-transformed high frequency HRV (log $msec^2$) during the stress challenge protocol, Mesa Stress 127

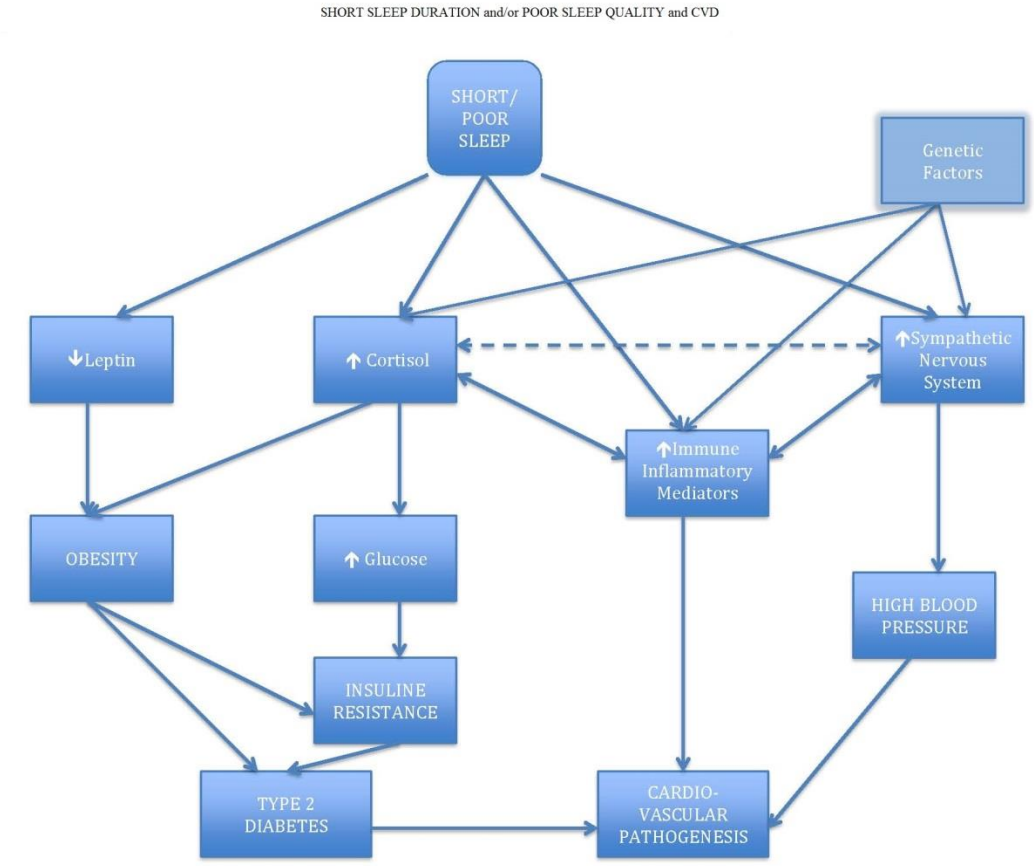
Supplemental Figure D-3 Mean log-transformed amylase (log U/mL) during the stress challenge protocol, MESA Stress 128

Supplemental Figure D-4 Mean log-transformed cortisol (log nmol/L) during the stress challenge protocol by sleep duration, sleep efficiency an insomnia, MESA Stress 129

Supplemental Figure D-5 Mean log-transformed cortisol (log nmol/L) during the stress challenge protocol by demographics, MESA Stress 130

APPENDIX A Chapter1

Supplemental Figure A-1 Probable mechanisms involved in the association of short/poor sleep and CVD outcomes



APPENDIX B CHAPTER 2

Supplemental Table B-1 Sleep duration and derived cortisol variables of salivary diurnal cortisol used in epidemiological studies

Author (Pub Year)	***Sleep Measure***		***Time Point***		***Deviation***		***AUC***		***Covariates***
	Subjective measures	***Objective measures Actigraphy/PSG***	***Measure***	***Significant Result***	***Measure***	***Significant Result***	***Measure***	***Significant Result***	
Ekstedt,[110] 2004 Sweden		Total Sleep Time	Mean value of all 4 morning samples	No association	Difference sample at 0 and 60 min after awakening				Gender Burnout
Hansen,[81] 2012 Denmark (Whole population)	Sleep Duration		Morning concentration (30 min after awakening) Evening cortisol (8pm)	No association	Morning-to evening change	No association			Sampling time, pain medication, age, BMI, Alcohol consumption Smoking Physical activity Education Chronic diseases Work schedule
Hansen,[81] 2012 Denmark (Subsample)	: Sleep duration		Morning concentration Evening cortisol (8pm)		CAR Slope	No association	AUC-morning	No association	Same as above
Kumari,[80] 2009 UK	Sleep duration				CAR Slope	Short sleep duration associated with an increase in CAR and shallow slope in diurnal cortisol			Age, sex, social position, employment grade, awakening time, smoking status, waist circumference, SF-36 physical and mental health)
Rueggeberg,[90] 2012 Canada	Sleep Duration (SD)						AUC (total diurnal cortisol)	Lower cortisol at baseline and 2 years levels of SD (compared to higher) were associated with increase in cortisol over the subsequent 2 years	Age, sex, partnership status, education, chronic illness, cortisol-released medication* use, BMI, smoking,

Author (Pub Year)	*Sleep Measure*		*Time Point*		*Deviation*		*AUC*		*Covariates*
	Subjective measures	***Objective measures Actigraphy/PSG***	***Measure***	***Significant Result***	***Measure***	***Significant Result***	***Measure***	***Significant Result***	
Vargas,[99] 2014 USA	Total sleep time (TST)		Awakening	Lower TST was associated with lower cortisol at awakening	CAR	Lower TST was associated with greater CAR			Age, sex, contraceptive use, medication use, sleep patterns, depressive symptoms, wake-up time differences**
Zhang,[115] 2011 China		Sleep duration	3-day average morning awakening cortisol	No association					

Footnote

*Antidepressants, beta blockers, anti-inflammatories

** difference between typical wake up time and actual wake-up time

Supplemental Table B-2 Sleep quality and indices of salivary diurnal cortisol and covariates used in epidemiological studies

Author (Pub Year)	*Sleep Measure*		*Time Point*		*Deviation*		*AUC*		*Covariates*
	Subjective measures	***Objective measures Actigraphy/PSG***	***Measure***	***Significant Result***	***Measure***	***Significant Result***	***Measure***	***Significant Result***	
Backhaus,[91] 2004 Germany	Sleep quality Sleep disturbance Sleep rumination		Mean cortisol values over 7 days): Awakening (T1), 15 min after awakening (T2), Before bedtime (T3)	Primary insomniacs had lower T1 than non-insomniacs. No difference in T3 between groups	Deviation: T1 – T3	T1 – T3 was smaller in insomniacs than in non-insomniacs			Cases (insomniacs) and controls did not differ with regard to age, sex, BMI, time of awakening or bedtime
Eek,[93] 2012 Sweden	Sleep Quality		Mean over one day: Concentrations at the three time points, Cortisol morning peak, Mean morning concentration	No associations	The relative awakening response (CAR) The cortisol decline over the day	No associations			Awakening time, age and gender
Ekstedt,[110] 2004 Sweden		Sleep quality: Sleep efficiency # of arousals/hour	Mean value of all 4 morning samples at awakening	Higher frequency of arousals were associated with higher morning salivary cortisol and at awake	Difference sample at 0 and 60 min after awakening				Gender Burnout
Garde,[96] 2011 Denmark	Sleep Quality: Disturbed sleep index (DSI) Awakening index		Maximum morning concentration Evening cortisol concentration	No association	CAR Decline (slope)	No association			Gender age

Author (Pub Year)	*Sleep Measure*		*Time Point*		*Deviation*		*AUC*		*Covariates*
	Subjective measures	***Objective measures Actigraphy/PSG***	***Measure***	***Significant Result***	***Measure***	***Significant Result***	***Measure***	***Significant Result***	
Hansen,[81] 2012 Denmark	Sleep quality past 4 weeks Sleep quality the night before Disturbed sleep Awakening problems		Morning concentration (30 min after awakening) Evening cortisol (8pm)	Decreased morning cortisol Decreased evening cortisol (poor sleep past 4 weeks)	Morning-to evening change	No association			Sampling time Pain medications Age BMI Alcohol consumption Smoking Physical activity Education Chronic diseases Work schedule
Hansen,[81] 2012 Denmark	Subsample: Sleep quality the night before		Morning concentration Evening cortisol (8pm)	Lower salivary cortisol	CAR slope	Reduced CAR Reduced slope	AUC-morning	No association	Same as above
Hanson,[98] 2000 Netherlands	Subjective sleep quality		Mean cortisol	No association					Time, regular mood
Kumari,[80] 2009 UK	Sleep disturbance				CAR slope	Sleep disturbance was associated with a shallow slope in cortisol secretion			Age, sex, employment grade, awakening time, smoking status, waist circumference, SF-36 physical and mental health)
Lasikiewicz,[97]2008, UK	Sleep quality		Diurnal mean		Diurnal decline (slope) Cluster 1: blunted CAR with flattened decline Cluster 2: Classical profile	Cluster 1 was associated with poorer sleep quality	AUC		For Slope: gender, BMI, subjective easy to sleep, sleep onset For diurnal mean: gender, SBP, DBP, wakefulness, time to wake

Author (Pub Year)	*Sleep Measure*		*Time Point*		*Deviation*		*AUC*		*Covariates*
	Subjective measures	***Objective measures Actigraphy/PSG***	***Measure***	***Significant Result***	***Measure***	***Significant Result***	***Measure***	***Significant Result***	
Rueggeberg,[90] 2012 Canada	Sleep quality						AUC (total diurnal cortisol)		Sociodemographics (age, se, partnership status and education) Chronic illness, medication usage, BMI, and smoking
Rydstedt,[113] 2013 UK	Sleep quality		Mean 7-day cortisol Awakening and 22:00 hr.	No association					Age, sex
Seelig,[92] 2013 Switzerland,		Sleep efficiency Number of arousals Sleep stages	Cortisol level at midnight and early morning	Midnight salivary cortisol levels were higher in insomniacs than in non-insomniacs					
Vargas,[99] 2014 USA	Sleep onset latency Wake after sleep onset (WASO) Wake time Sleep Quality		Awakening	No association					Age, sex, contraceptive use medication, habitual sleep problems
Zhang,[115] 2011 China		Sleep duration Sleep efficiency	3-day average morning awakening cortisol	No association					Age, Gender, BMI, education level, waist/hip ratio, systolic and diastolic blood pressure and creatinine
Zhang,[95] 2014 China		Sleep efficiency					AUC of CAR AUCg (ground) AUCi (increase) Diurnal cortisol (slope) Time points	Insomnia was associated with increased CAR activity Poor subjective sleep quality was associated with higher evening cortisol levels in the insomnia group	Age, sex, BMI, waist-to hip ratio, current depressive disorder, current anxiety disorder, chronic medication use, chronic medical condition, bedtime, wake time at the morning, and seasonality

Supplemental Table C-1 First sample time (in minutes) since self-reported wake up after deleting exclusions, MESA Study

day	Max	P95	P90	Q3	Median	Q1	P10	P5	Min
1	185	45	30	10	4	1	0	0	0
2	298	46	30	11	5	1	0	0	0

Supplemental Table C-2 Number of samples per subjects (after exclusions), MESA Study

No of Samples per Subject	Count of Subjects	%
2	1	0.1
4	1	0.1
6	1	0.2
7	1	0.2
8	2	0.3
9	1	0.2
10	4	0.7
11	8	1.3
12	19	3.2
13	33	5.5
14	91	15.2
15	126	21.0
16	312	52.0
No. of Total Subjects	600	100.0%

Supplemental Table C-3 Number of samples per participant day (after exclusions)

DAY	No. of Samples per Day	Participant Days*	%
Day 1	1	2	0.3
	2	2	0.3
	4	3	0.5
	5	13	2.2
	6	29	4.8
	7	151	25.2
	8	399	66.7
Total participant days in Day 1		599	100.0%
Day 2	3	2	0.3
	4	4	0.7
	5	15	2.5
	6	43	7.2
	7	156	26.0
	8	379	63.3
Total participant days in Day 2		599	100.0%
No. of Total Days		**1198**	

*For instance, on day 1, only 2 participants provided one sample for the whole day whereas 399 participants provided 8 samples for the whole day.

Supplemental Table C-4 Distribution of salivary sample collection time (hours) since awakening in MESA

Sample	5th Pctl	10th Pctl	Lower Quartile	Median	Upper Quartile	90th Pctl	95th Pctl
1	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00
2	0.56	0.53	0.55	**0.51**	0.52	0.56	0.51
3	2.70	2.71	2.82	**2.67**	2.68	2.84	3.07
4	5.01	4.59	3.95	**3.40**	3.50	3.82	4.15
5	6.82	6.46	5.91	**5.40**	5.25	5.35	5.74
6	11.00	10.59	9.90	**9.27**	8.92	8.69	8.68
7	12.70	12.42	11.90	**11.31**	11.00	10.90	11.15
8	12.85	13.65	14.62	**15.15**	15.29	14.95	14.65

Supplemental Table C-5 Characteristics of cortisol data collection by demographic characteristics from MESA Stress participants

	Percent distribution of participants	Percent of samples with reported times within 15 min of wake-up	Percent of samples with reported times within 5 min of wake-up	First sample time (median)	Difference between first and second sample time (Q1 – Q3)
All subjects (n = 600)	100.0%	84.6	63.3	6:13	0:30 – 0.32
Age					
54-64	34.7%	86.0%	60.4%	6:11	0:30 – 0.32
65-74	34.9%	81.6%	50.2%	6:13	0:30 – 0.32
75-84	25.3%	80.1%	51.0%	6:15	0:30 – 0.32
≥85	5.0%	63.3%	50.0%	7:10	0:28 – 0.33
p-Value		*0.022*	*0.150*	*0.137*	*0.6384*
Sex					
Fem	53.0%	79.9%	54.1%	6:17	0:30 – 0.32
Male	47.0%	84.0%	53.7%	6:12	0:30 – 0.32
p-Value		*0.201*	*0.922*	*0.006*	*0.1944*
Race/Ethnicity					
White	27.7%	87.2%	64.0%	6:15	0:30 – 0.33
Black	32.3%	68.4%	37.4%	6:15	0:30 – 0.33
Hispanic	40.0%	88.8	60.2%	6:13	0:30 – 0.31
p-Value		*<0.0001*	*<0.0001*	*0.196*	*0.7323*
Income-wealth index					
0-1 points	13.7%	81.3%	51.3%	7:00	0:29 – 0.35
2-3 points	24.8%	83.0%	57.5%	6:15	0:30 – 0.31
4-6 points	42.3%	81.1%	52.9%	6:13	0:30 – 0.32
7-8 points	19.2%	85.5%	57.3%	6:13	0:30 – 0.33
p-Value		*0.778*	*0.705*	*0.944*	*04648*
Sleep Duration					
6 hours or less	33.7%	77.5%	47.0%	6:13	0:30 – 0.32
6 to 7 hours	33.3%	82.1%	54.6%	6:13	0:30 – 0.32
7 hours or more	33.0%	85.9%	60.3%	7:00	0:30 – 0.32
p-Value		*0.091*	*0.028*	*0.003*	*0.2769*
Sleep Efficiency					
Less than 85%	10.1%	81.4	57.6	6:15	0:30 – 0.32
85% or more	89.9%	81.9	53.5	6:13	0:30 – 0.32
p-Value		*0.918*	*0.550*	*0.300*	*0.5368*

Supplemental Table C-6 Characteristics of participants (n = 600) by mean sleep duration in hours (mean (SD)), MESA Study (2010-2012)

Characteristics	Number of participants	Sleep Duration (hours)		*p-value**
		Mean	SD	
Overall	600	6.44	1.2	
Age category, years				*0.2*
54 – 64	207	6.42	1.1	
65 – 74	211	6.42	1.2	
75 – 84	152	6.38	1.2	
≥ 85	30	6.96	1.2	
Sex (% distribution)				**0.001**
Female	316	6.58	1.2	
Male	284	6.27	1.2	
Race/ethnicity (% distribution)				**< 0.0001**
White	166	6.81	1.1	
Black	191	6.01	1.2	
Hispanic	243	6.51	1.2	
Study site (% distribution)				*0.1*
Columbia	265	6.32	1.2	
Johns Hopkins	162	6.51	1.1	
UCLA	173	6.54	1.2	
Education (% distribution)				*0.8*
Less than High School to GED	238	6.44	1.2	
Some College to Associate Degree	181	6.39	1.2	
Bachelor or greater	179	6.48	1.2	
Mean income (in thousands USD)				***<0.01***
Lowest tertile	184	6.47	1.2	
Second tertile	180	6.21	1.2	
Highest tertile	232	6.59	1.1	
Income-Wealth Index (% distribution)				***0.05***
0 − 1	74	6.17	1.2	
2 − 3	124	6.48	1.2	
4 − 6	222	6.33	1.2	
7 − 8	116	6.59	1.1	
Marital status				*0.4*
Married/Living with partner	328	6.49	1.1	
Living without partner	259	6.41	1.2	
Body Mass Index (% distribution)				***<0.0001***
Normal	118	6.69	1.1	
Obesity Grade 1	238	6.54	1.2	
Obesity Grade 2	221	6.26	1.2	
Obesity Grade 3	23	5.68	1.1	
Smoking status (% distribution)				*0.3*
Never	327	6.44	1.2	
Former and quit > 1 year ago	222	6.48	1.2	
Current and former quit ≤ 1 year ago	48	6.18	1.2	
Alcohol Use Presently				*0.6*
Yes	239	6.47	1.2	
No	360	6.42	1.2	

Supplemental Table, continued

Characteristics	Number of participants	Sleep Duration (hours) Mean	Sleep Duration (hours) SD	*p-value**
Hypertension (by JNC VI, 1997)				*0.4*
Yes	358	6.40	1.2	
No	242	6.49	1.1	
Diabetes (% distribution)				***<0.05***
Yes	248	6.32	1.2	
No	348	6.52	1.2	
Depression CESD >16 (% distribution)				*0.5*
Yes	97	6.36	1.3	
No	492	6.46	1.1	
Hostility (% distribution)				*0.2*
Lowest tertile	188	6.54	1.0	
Second tertile	238	6.39	1.2	
Highest tertile	153	6.31	1.3	
Use of Oral Steroids (%)				*0.4*
Yes	8	5.95	1.7	
No	592	6.44	1.2	
Use of Inhaled Steroids (%)				***<0.01***
Yes	15	5.57	1.0	
No	585	6.46	1.2	
Use of any Antihypertensive Med (%)				*0.1*
Yes	340	6.37	1.2	
No	260	6.53	1.1	
Use of HRT (%)				*0.1*
Yes	18	6.90	1.0	
No	582	6.42	1.2	
Use of any Antidepressant (%)				***0.01***
Yes	66	6.77	1.3	
No	534	6.39	1.2	
Any insomnia symptom (%)				*0.3*
Yes	209	6.37	1.2	
No	382	6.48	1.1	
All apneas and hypopneas**				*0.2*
AHI ≥ 15	323	6.38	1.2	
AHI < 15	218	6.53	1.1	
Mean (SD) Sleep efficiency (%)				***<0.0001***
Efficiency < 85%	60	5.71	1.1	
Efficiency ≥ 85%	540	6.51	1.2	

* t-test or ANOVA test or Kruskall Wallis

** apnea (all apneas and hypopnea per hour of the sleep with ≥ 3% or greater desaturation – index (AHI))

Supplemental Table C-7 Percent differences (95% confidence intervals) in features of the daily cortisol associated with sleep duration (≥3 hours to <9 hours) and sleep efficiency (%), specified as continuous, MESA Study

	Sleep Duration[a] (1 hour less)	Sleep Efficiency[b] (1% less)
Percent difference in wake-up (CI 95%)		
Model 1[c]	−5.67 (−12.7, 0.9)	−1.52 (−4.1, 1.0)
Model 2[d]	−4.67 (−12.5, 2.6)	−1.84 (−4.7, 0.9)
Model 3[e]	−4.23 (−12.3, 3.2)	−2.01 (−4.9, 0.8)
Model 4[f]	−2.68(−10.3, 4.5)	−1.80 (−4.7, 1.0)
Percent difference in cortisol awakening response		
Model 1[c]	−3.10 (−10.3, 3.6)	−0.39 (−2.9, 2.0)
Model 2[d]	−2.40 (−10.2, 4.8)	−0.16 (−2.9, 2.5)
Model 3[e]	−3.13 (−11.4, 4.5)	−0.10 (−2.9, 2.6)
Model 4[f]	−3.27 (−11.0, 3.9)	0.13 (−2.5, 2.8)
Percent difference in early decline		
Model 1[c]	2.08 (−3.5, 8.0)	**1.84 (0.1, 3.6)**§
Model 2[d]	0.65 (−5.2, 6.8)	**1.98 (0.3, 3.7)**§
Model 3[e]	−0.06 (−6.5, 5.9)	**2.09 (0.3, 3.9)**§
Model 4[f]	−1.92 (−8.5, 4.3)	**2.23 (0.4, 4.1)**§
Percent difference in late decline		
Model 1[c]	**0.92 (0.4, 1.5)****	−0.08 (−0.2, 0.1)
Model 2[d]	**1.02 (0.5, 1.6)****	−0.09 (−0.2, 0.1)
Model 3[e]	**1.13 (0.6, 1.7)*****	−0.08 (−0.2, 0.1)
Model 4[f]	**1.28 (0.7, 1.9)*****	−0.17 (−0.3, 0.0)
Summary measures		
Percent difference in wake-to-bed slope		
Model 1[c]	**0.99 (0.52, 1.46)*****	−0.06 (−0.07, 0.19)
Model 2[d]	**1.00 (0.50, 1.50)*****	0.07 (−0.07, 0.20)
Model 3[e]	**1.02 (0.50, 1.54)*****	0.08 (−0.05, 0.22)
Model 4[f]	**1.01 (0.47, 1.55)****	0.01 (−0.14, 0.15)
Percent difference in AUC		
Model 1[c]	−0.06 (−5.23, 4.85)	0.16 (−1.33, 1.68)
Model 2[d]	0.22 (−5.13, 5.88)	0.20 (−1.45, 1.87)
Model 3[e]	−0.36 (−6.05, 5.02)	0.29 (−1.41, 2.01)
Model 4[f]	−0.65 (−6.58, 4.94)	0.34 (−1.42, 2.14)

§<0.05; *≤0.01; **≤0.001 ; ***≤0.0001

[a] Sleep duration was estimated as continuous (hours)
[b] Sleep efficiency was estimated as percentage (%)

Models:
[c] Model 1 : Model adjusted for day of saliva collection, time of wake-up, gender, age, race/ethnicity, and income-wealth index
[d] Model 2 : Model 1 + body mass index, smoking, alcohol consumption, medications (oral and inhaled steroids, hormone replacement therapy and antidepressants) and apnea/hypopnea index (AHI)
[e] Model 3 : Model 2 + hypertension, diabetes, and depression
[f] Model 4 : Model 3 + sleep efficiency (as continuous) or sleep duration (as continuous) in models for sleep duration and sleep efficiency, respectively

Supplemental Table C-8 Percent differences (95% confidence intervals) in features of the daily cortisol associated with sleep duration specified as dichotomized and continuous when participants were restricted to sleep duration < 8 hours

	Sleep Duration	
	Dichotomized[a] (<6 hr vs. ≥6 hr)	Continuous[b] (1 hour less)
Percent difference in wake-up (CI 95%)		
Model 1[c]	-12.08 (-31.2, 4.3)	−6.94 (−14.8, 0.4)
Model 2[d]	−13.25 (−35.1, 5.0)	−5.924 (−14.5, 2.0)
Model 3[e]	−12.03 (−34.3, 6.5)	−5.80 (−14.8, 2.5)
Model 4[f]	−9.16 (−30.4, 8.6)	−3.99 (−12.5, 3.9)
Percent difference in cortisol awakening response		
Model 1[c]	−8.76 (−25.5, 5.8)	−3.97 (−12.7, 4.1)
Model 2[d]	−8.35 (−27.1, 7.6)	−3.64 (−13.1, 5.01)
Model 3[e]	−9.74 (−29.2, 6.8)	−4.80 (−14.81, 4.31)
Model 4[f]	−9.67 (−28.3, 6.3)	−4.85 (−14.3, 3.81)
Percent difference in early decline		
Model 1[c]	7.55 (−3.9, 20.3)	4.38 (−1.95, 11.1)
Model 2[d]	8.62 (−4.1, 23.1)	4.49 (−2.45, 11.9)
Model 3[e]	8.14 (−5.0, 23.0)	3.95 (−3.4, 11.89)
Model 4[f]	4.82 (−7.6, 19.0)	1.81 (−5.44, 9.6)
Percent difference in late decline		
Model 1[c]	**1.55 (0.3, 2.8)**[§]	**0.63 (0.0, 1.2)**[§]
Model 2[d]	**1.62 (0.2, 3.0)**[§]	0.61 (−0.0, 1.2)
Model 3[e]	**1.57 (0.2, 3.0)**[§]	0.66 (−0.0, 1.3)
Model 4[f]	**1.79 (0.4, 3.2)**[§]	**0.81 (0.1, 1.5)**[§]
Percent difference in wake-to-bed slope		
Model 1[c]	**1.93 (0.8, 3.0)**[**]	**0.88 (0.3, 1.4)**[*]
Model 2[d]	**2.14 (1.0, 3.3)**[**]	**0.89 (0.3, 1.5)**[*]
Model 3[e]	**2.03 (0.8, 3.2)**[**]	**0.87 (0.3, 1.5)**[*]
Model 4[f]	**1.99 (0.8, 3.2**[**]	**0.85 (0.2, 1.5)**[*]

[§]<0.05; [*]≤0.01; [**]≤0.001 ; [***]≤0.0001

[a] Sleep duration was estimated as dichotomized (short sleep duration (<6hours)) vs. longer sleep duration (reference, ≥ 6hours to < 8 hours)

[b] Sleep duration was estimated as continuous (3 to < 8 hours)

Models:

[c] Model 1 : Model adjusted for day of saliva collection, time of wake-up, gender, age, race/ethnicity, and income-wealth index

[d] Model 2 : Model 1 + body mass index, smoking, alcohol consumption, medications (oral and inhaled steroids, hormone replacement therapy and antidepressants) and apnea/hypopnea index (AHI)

[e] Model 3 : Model 2 + hypertension, diabetes, and depression

[f] Model 4 : Model 3 + sleep efficiency (as continuous)

Supplemental Table C-9 Percent differences (95% confidence intervals) in wake−to−bed slope of salivary cortisol level associated with sleep duration dichotomized at 5 hours (first column) and at 7 hours (second column) for the overall sample and when restricted, MESA

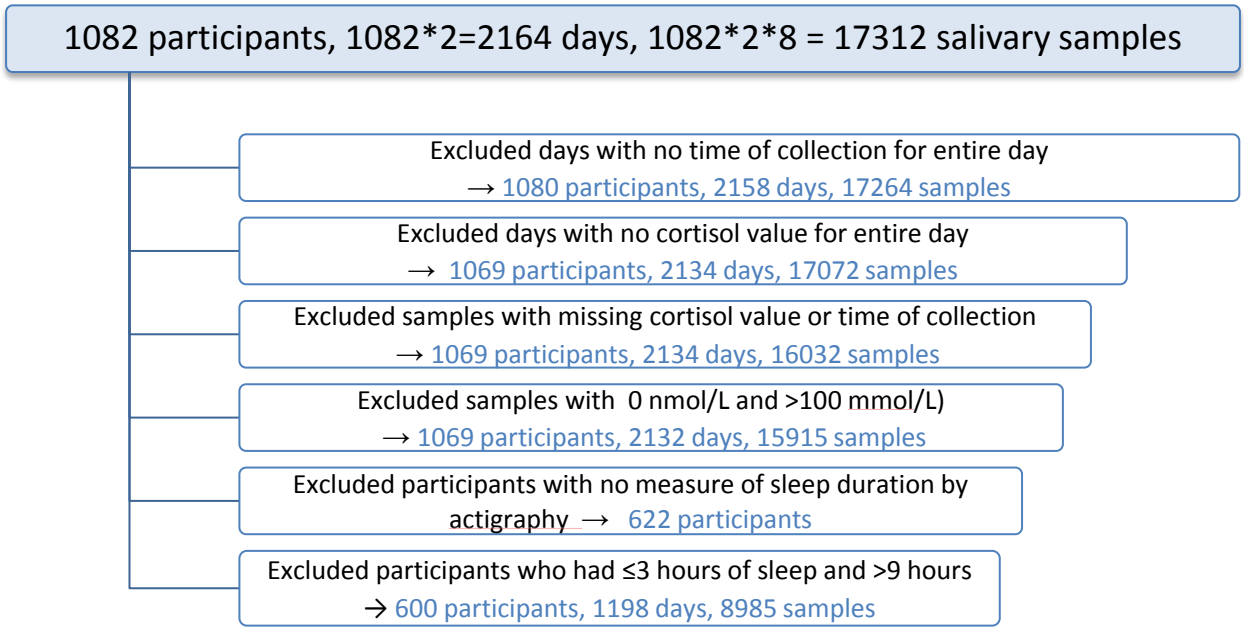
	Sleep Duration Dichotomized[a]	
	cut off 5 HRS	***cut off 7HRS***
Whole sample		
Model 1[c]	1.08 (−0.7, 2.9)	2.04 (1.1, 3.0)***
Model 2[d]	0.93 (−0.9, 2.8)	2.11 (1.1, 3.1)***
Model 3[e]	0.75 (−1.2, 2.7)	2.28 (1.3, 3.3)***
Model 4[f]	0.59 (−1.4, 2.6)	2.22 (1.2, 3.3)***
Restricted to 8 hours (≥ 3 to < 8 hrs)[b]		
Model 1[c]	0.9 (−0.8, 2.7)	1.65 (0.6, 2.7)**
Model 2[d]	0.8 (−1.1, 2.7)	1.75 (0.7, 2.8)**
Model 3[e]	0.6 (−1.3, 2.6)	1.85 (0.8, 2.9)**
Model 4[f]	0.5 (−1.5, 2.5)	1.80 (0.7, 2.9)**

*≤ 0.01 **≤ 0.001 ***≤ 0.0001

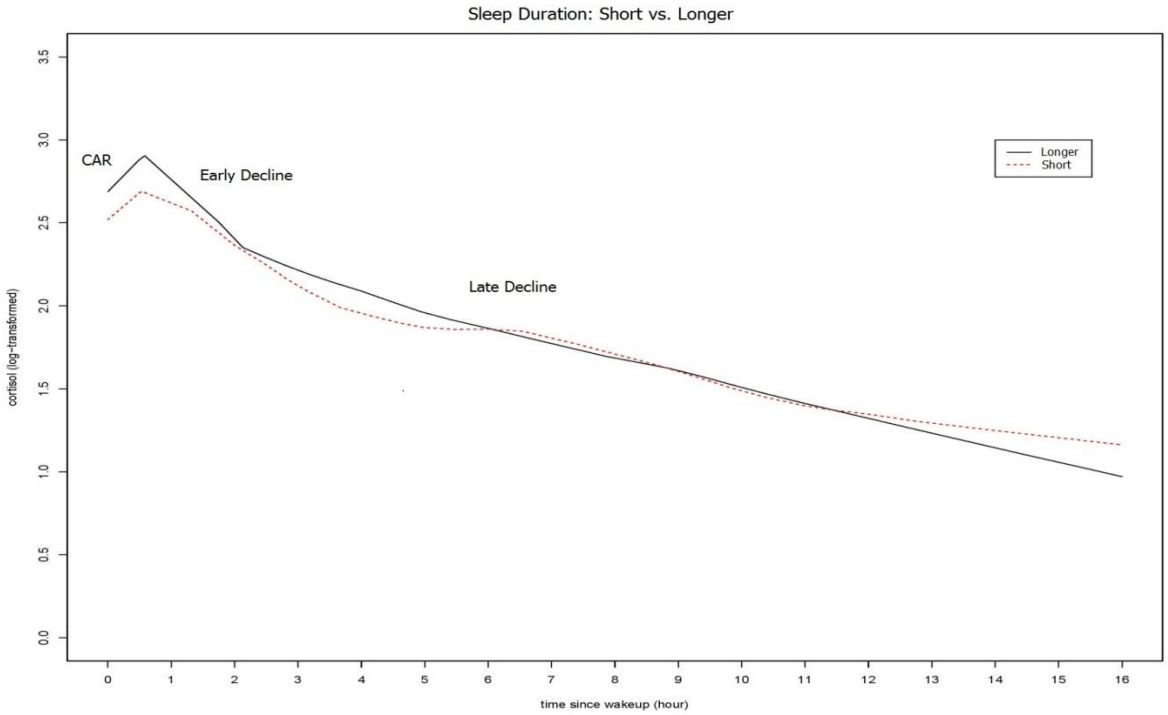
[a] Sleep duration was dichotomized (short sleep duration (<5 or <7 hours,) vs. longer (reference, ≥ 5, or ≥7 hours, respectively).
[b] Sample restricted to < 8 hours of sleep duration

Models:
[c] Model 1 : Model adjusted for day of saliva collection, time of wake−up, gender, age, race/ethnicity, and income−wealth index
[d] Model 2 : Model 1 + body mass index, smoking, alcohol consumption, medications (oral and inhaled steroids, hormone replacement therapy and antidepressants) and apnea/hypopnea index (AHI)
[e] Model 3 : Model 2 + hypertension, diabetes, and depression
[f] Model 4 : Model 3 + sleep efficiency (as continuous)

Supplemental Figure C-1 Chart of flow of exclusion of participants, days or salivary samples



Supplemental Figure C-2 LOESS Plot log transformed cortisol by sleep duration

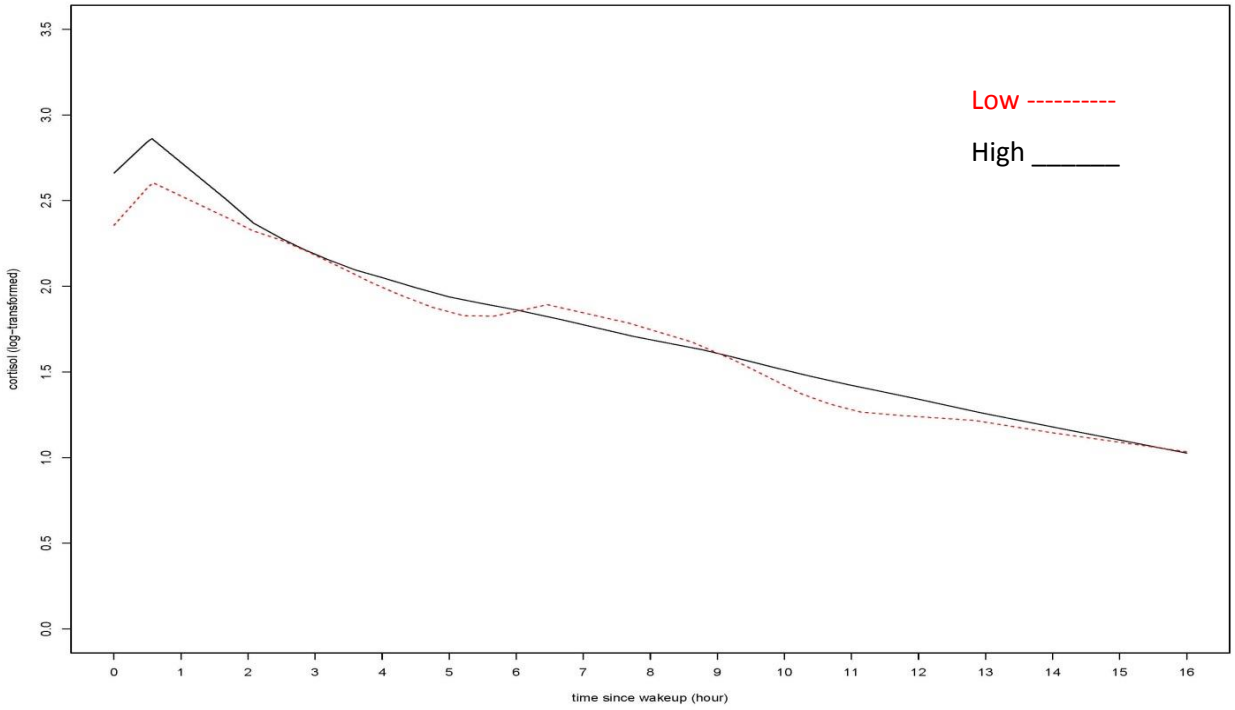


Note: Short sleepers had lower cortisol at awakening and at 30 minutes after as well as slower decline later in the day and higher levels of cortisol at bedtime than longer sleepers.

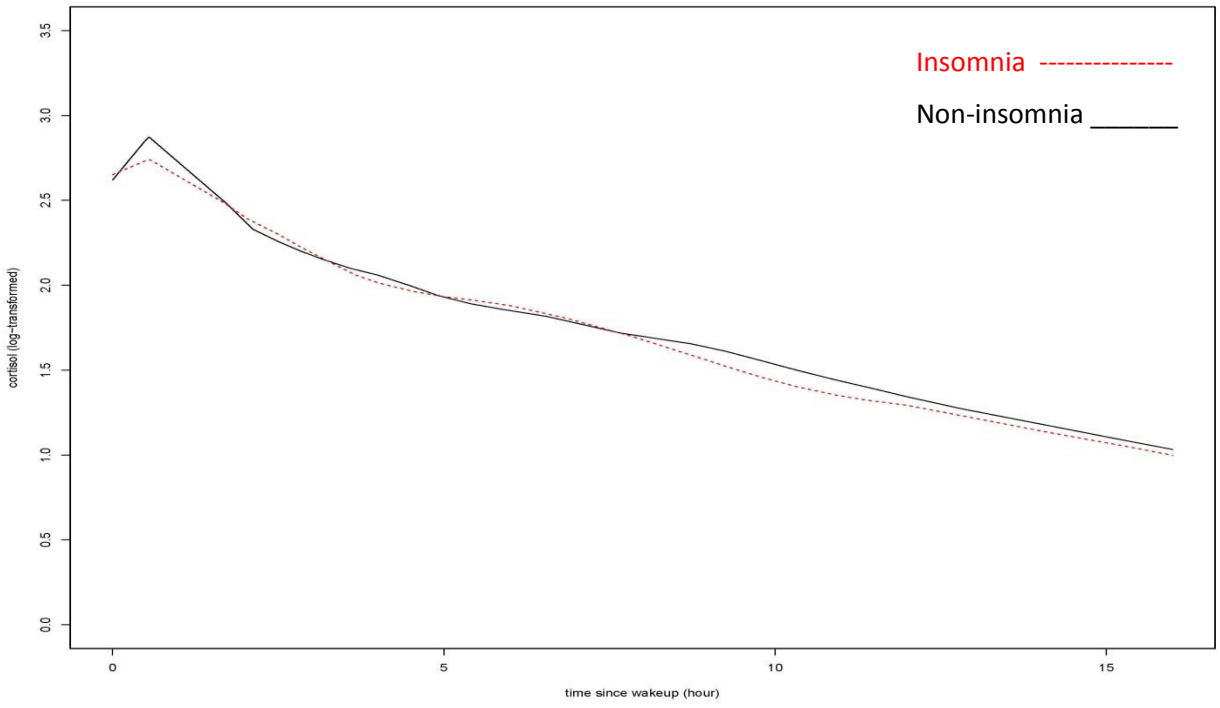
(CAR = Cortisol awakening response)

Supplemental Figure C-3 LOESS plot log-transformed cortisol by sleep efficiency and insomnia

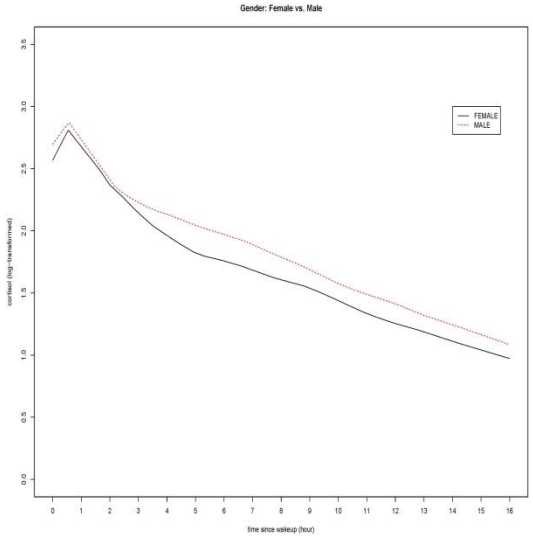
Sleep Efficiency: Low vs. High

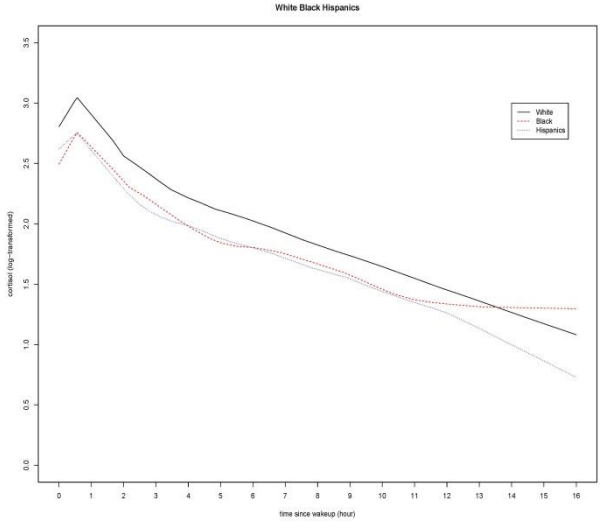


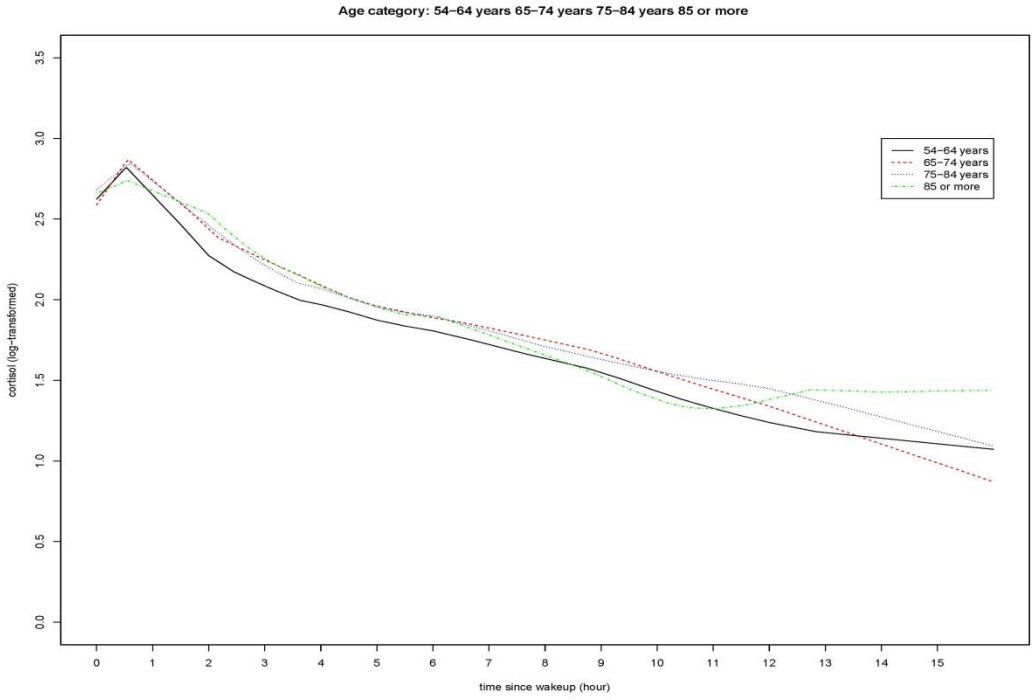
Subjective Sleep Quality: Insomnia vs. Non-insomnia



Supplemental Figure C-4 LOESS plot log-transformed cortisol by gender, race/ethnicity and age







APPENDIX D Chapter 4

Supplemental Table D-1 Characteristics of participants (n= 527) by sleep duration at < 6 hours, between 6 and less than 7 hours and 7 or more hours, MESA

	Sleep Duration (hours)¶			
	< 6 hours (n=173)	6 to < 7 hours (n=185)	≥7 hours (n=169)	P*
	N(%) or Mean ± SD)			
Demographics				
Age (years)	67.7±9.2	68.1±8.3	69.0±8.9	.44
Male	86(50)	91(49)	65 (39)	.06
Race/ethnicity				
White	27 (16)	48 (26)	68 (40)	**<.0001**
Black	78 (45)	59 (32)	26 (15)	
Hispanic	68 (39)	78 (42)	75 (44)	
Income-Wealth Index	3.9±2.2	4.5±2.2	4.4±2.3	**.03**
Lifestyle Characteristics				
BMI	30.7±5.8	29.6±5.1	28.1±4.6	**<.0001**
Current smokers	19 (11)	14 (8)	11 (7)	.52
Alcohol consumption	65 (38)	74 (40)	76 (45)	.39
Hypertension	106 (61)	103 (56)	99 (59)	.56
Diabetes	67 (39)	84 (46)	58 (35)	.10
Depression CESD >16	36 (21)	23 (13)	25 (15)	.08
Steroids[1] users	14 (8)	2 (1)	3 (2)	**<.001**
Antihypertensive users	104 (60)	99 (54)	93 (55)	.42
Hormone therapy users	4 (2)	7 (4)	9 (5)	.35
Antidepressant users [2]	15 (9)	17 (9)	27 (16)	.06
Sleep Variables				
Sleep efficiency <85%	29 (17)	20 (11)	5 (3)	**<.0001**
Insomnia symptom[3]	68 (40)	58 (32)	58 (35)	.25
AHI[4] ≥ 15	93(62)	96(56)	88(57)	.52
Naps[5] (min/day)	61±51	34±33	30±34	**<.0001**

¶ Sleep duration in this sample were between > 3 hours and < 9 hours

*either chi-square or t-test/ANOVA or Kruskall Wallis test for variables non-normally distributed

[1] oral and inhalers

[2] including medication for sleep and mood (including benzodiazepines)

[3] WHIIRS score ≥ 9 = insomnia

[4]AHI = all apneas and hypopnea per hour of the sleep with ≥ 3% or greater desaturation – index;

[5]all average sleep time in naps per day across all days when only naps with >=15 minutes of sleep time are counted

Supplemental Table D-2 Mean Value* log-transformed heart rate (log (beats/min)) at baseline and responses to the stress challenge by sleep duration (SD), sleep efficiency (SE) and insomnia

Heart Rate (HR)	**Long SD (>= 6 hrs)**	**Short SD (<6 hrs)**		**Long SD (>= 7 hrs)**	**Short SD (<7 hrs)**	
	N= 354	N = 173	**Difference**	N = 169	N = 358	**Difference**
	Mean (SD)	Mean (SD)		Mean (SD)		Mean (SD)
HR at baseline	4.20 (0.15)	4.22 (0.17)	0.02	4.20 (0.15)	4.21 (0.16)	0.01
HR at Mental Stress Task	4.25 (0.15)	4.27 (0.16)	0.03	4.24 (0.15)	4.26 (0.16)	0.03
HR at Recovery	4.21 (0.15)	4.24 (0.17)	0.03	4.21 (0.15)	4.23 (0.16)	0.02
HR at Orthostatic	4.27 (0.16)	4.31 (0.17)	0.04	4.26 (0.15)	4.29 (0.17)	0.03
Reactivity to Mental Stress Task[1]	0.04 (0.05)	0.05 (0.05)	0.01	0.04 (0.05)	0.05 (0.05)	0.01
Recovery from Stress Task[2]	-0.04 (0.04)	-0.04 (0.04)	0.00	-0.03 (0.04)	-0.04 (0.04)	0.00
Orthostatic Reactivity[3]	0.07 (0.06)	0.08 (0.06)	0.01	0.07 (0.06)	0.07 (0.06)	0.01
	High SE (>= 85%)	**Low SE (< 85%)**		**Non-Insomnia**	**Insomnia**	
	N = 473	N = 54	**Difference**	N = 334	N = 184	**Difference**
	Mean (SD)	Mean (SD)		Mean (SD)	Mean (SD)	
HR at baseline (beats/ min)	4.21 (0.15)	4.23 (0.18)	0.02	4.20 (0.16)	4.22 (0.16)	0.02
HR at Mental Stress Task (beats/min)	4.25 (0.15)	4.28 (0.18)	0.02	4.25 (0.15)	4.27 (0.16)	0.02
HR at Recovery (beats/min)	4.22 (0.15)	4.24 (0.18)	0.02	4.21 (0.15)	4.23 (0.16)	0.02
HR at Orthostatic (beats/min)	4.28 (0.16)	4.31 (0.19)	0.03	4.27 (0.16)	4.29 (0.16)	0.02
Reactivity to Mental Stress Task[1]	0.04 (0.05)	0.04 (0.05)	0.00	0.04 (0.05)	0.04 (0.05)	0.00
Recovery from Stress Task[2]	-0.04 (0.04)	-0.03 (0.04)	0.00	-0.04 (0.04)	-0.03 (0.04)	0.00
Orthostatic Reactivity[3]	0.07 (0.06)	0.06 (0.05)	-0.01	0.07 (0.06)	0.07 (0.06)	0.00

**based on log-transformed HR (unit: log(beats/min)) without any adjustments*

[1]Reactivity to Mental Stress Task = HR at mental stress task – HR at baseline
[2]Recovery from Stress Task = HR at recovery – HR at mental stress task
[3]Orthostatic Reactivity = HR at orthostatic – HR at baseline

Supplemental Table D-3 Mean value* log-transformed high frequency HR variability (log(msec^2)) at baseline and responses to the stress by sleep duration (SD), sleep efficiency (SE) and insomnia

High Frequency HRV (HF-HRV)	Long SD (>= 6 hrs)	Short SD (<6 hrs)	Difference	Long SD (>= 7 hrs)	Short SD (<7 hrs)	Difference
	N= 354	N = 173		N = 169	N = 358	
HF-HRV at baseline	4.75 (1.29)	4.88 (1.51)	0.14	4.91 (1.25)	4.73 (1.42)	-0.18
HF-HRV at Mental Stress Task	4.37 (1.26)	4.42 (1.47)	0.05	4.58 (1.19)	4.30 (1.39)	-0.29
HF-HRV at Recovery	4.67 (1.31)	4.74 (1.40)	0.07	4.78 (1.21)	4.65 (1.39)	-0.12
HF-HRV at Orthostatic	4.08 (1.40)	4.07 (1.51)	-0.01	4.23 (1.50)	4.00 (1.40)	-0.23
Reactivity to Mental Stress Task[1]	-0.35 (0.75)	-0.40 (0.77)	-0.05	-0.31 (0.77)	-0.40 (0.75)	-0.09
Recovery from Stress Task[2]	0.28 (0.69)	0.32 (0.64)	0.04	0.20 (0.65)	0.34 (0.68)	0.14
Orthostatic Reactivity[3]	-0.69 (0.92)	-0.74 (0.93)	-0.05	-0.73 (0.99)	-0.69 (0.90)	0.04
	High SE (>= 85%)	**Low SE (< 85%)**	**Difference**	**Non-Insomnia**	**Insomnia**	**Difference**
	N = 473	N = 54		N = 334	N = 184	
HF-HRV at baseline	4.85 (1.28)	4.28 (1.89)	-0.57	4.79 (1.34)	4.82 (1.42)	0.03
HF-HRV at Mental Stress Task	4.46 (1.28)	3.74 (1.57)	-0.72	4.43 (1.34)	4.33 (1.32)	-0.10
HF-HRV at Recovery	4.76 (1.29)	4.12 (1.57)	-0.64	4.73 (1.35)	4.67 (1.31)	-0.06
HF-HRV at Orthostatic	4.17 (1.40)	3.32 (1.58)	-0.84	4.14 (1.48)	3.95 (1.36)	-0.19
Reactivity to Mental Stress Task[1]	-0.37 (0.75)	-0.38 (0.77)	-0.02	-0.34 (0.72)	-0.43 (0.83)	-0.08
Recovery from Stress Task[2]	0.29 (0.68)	0.34 (0.56)	0.04	0.28 (0.68)	0.33 (0.68)	0.05
Orthostatic Reactivity[3]	-0.71 (0.95)	-0.68 (0.65)	0.02	-0.64 (0.84)	-0.85 (1.06)	-0.21

** based on log-transformed HF-HRV (unit: log (sec^2))without any adjustments*

[1]Reactivity to Mental Stress Task = HF-HRV at mental stress task – HF-HRV at baseline
[2]Recovery from Stress Task = HF-HRV at recovery – HF-HRV at mental stress task
[3]Orthostatic Reactivity = HF-HRV at orthostatic – HF-HRV at baseline

Supplemental Table D-4 Mean values* of amylase (log(U/mL)) at each sample, reactivity and recovery by sleep duration (SD), sleep efficiency (SE) and insomnia.

	Long SD (>= 6 hrs)	**Short SD (<6 hrs)**	**Difference**	**Long SD (>= 7 hrs)**	**Short SD (<7 hrs)**	**Difference**
	N= 293	N = 158		N = 134	N = 317	
Amylase value at baseline[1]	4.26 (1.32)	4.31 (1.26)	0.05	4.19 (1.36)	4.31 (1.28)	0.12
Amylase value at 2nd sample	4.42 (1.20)	4.66 (1.10)	0.23	4.38 (1.24)	4.56 (1.14)	0.17
Amylase value at 3rd sample	4.27 (1.17)	4.46 (1.06)	0.20	4.24 (1.17)	4.38 (1.12)	0.13
Amylase value at 4th sample	4.36 (1.16)	4.42 (1.31)	0.07	4.30 (1.24)	4.42 (1.20)	0.12
Mental Stress Reactivity[2]	0.16 (0.95)	0.29 (0.77)	0.13	0.22 (0.79)	0.20 (0.94)	-0.02
Orthostatic Reactivity[3]	0.04 (0.96)	0.15 (0.82)	0.11	0.16 (1.13)	0.05 (0.81)	-0.11
Recovery from Stress Task[4]	-0.07 (0.92)	-0.23 (0.82)	-0.15	-0.10 (0.87)	-0.14 (0.90)	-0.04
	High SE (>= 85%)	**Low SE (< 85%)**	**Difference**	**Non-Insomnia**	**Insomnia**	**Difference**
	N = 405	N = 46		N = 284	N = 160	
Amylase value at baseline[1]	4.25 (1.31)	4.56 (1.17)	0.31	4.28 (1.32)	4.27 (1.27)	-0.01
Amylase value at 2nd sample	4.47 (1.19)	4.78 (0.89)	0.31	4.50 (1.14)	4.51 (1.22)	0.01
Amylase value at 3rd sample	4.32 (1.15)	4.49 (0.94)	0.17	4.34 (1.14)	4.33 (1.12)	0.00
Amylase value at 4th sample	4.36 (1.21)	4.57 (1.25)	0.21	4.37 (1.22)	4.40 (1.20)	0.04
Mental Stress Reactivity[2]	0.22 (0.93)	0.11 (0.57)	-0.11	0.21 (0.90)	0.20 (0.90)	0.00
Orthostatic Reactivity[3]	0.10 (0.92)	-0.11 (0.89)	-0.21	0.06 (0.92)	0.11 (0.91)	0.05
Recovery from Stress Task[4]	-0.12 (0.89)	-0.19 (0.86)	-0.07	-0.14 (0.92)	-0.09 (0.84)	0.05

**based on log-transformed Amylase (unit: log(nmol/L)) without any adjustment*

[1] Sample 1(baseline)

[2] Mental Stress Reactivity = Sample 2 – Sample 1

[3] Orthostatic Reactivity = Sample 3 – Sample 1

[4] Recovery from Stress Task = Sample 4 – Sample 2

Supplemental Table D-5 Mean differences in log transformed heart rate (log (beats/min)) at baseline and mean differences in responses to challenge by sleep duration, sleep efficiency and insomnia symptoms (N = 3450 observations, 527 persons)

	Sleep Duration*						**Sleep Efficiency**			**Insomnia Symptoms**		
	(< 6 hrs vs. ≥ 6 hrs)			**(< 7 hrs vs. ≥ 7 hrs)**			**low vs. high**			**Insomnia vs. non-insomnia**		
	Estimate	95% C.I.		Estimate	95% C.I.		Estimate	95% C.I.		Estimate	95% C.I.	
Baseline												
Model 1	0.0255	-0.0056	0.0566	0.0269	-0.0024	0.0561	0.0255	-0.0248	0.0759	0.0122	-0.0174	0.0417
Model 2	0.0289	-0.0021	0.0598	0.0252	-0.0047	0.0551	0.0139	-0.0361	0.0639	0.0106	-0.0187	0.0400
Model 3	**0.0360**	**0.0033**	**0.0686**	0.0280	-0.0036	0.0596	0.0112	-0.0411	0.0635	0.0046	-0.0265	0.0357
Model 4	**0.0404**	**0.0070**	**0.0739**	0.0287	-0.0033	0.0606	0.0096	-0.0427	0.0620	0.0060	-0.0247	0.0367
Reactivity to Mental Stress[1]												
Model 1	0.0075	-0.0030	0.0180	0.0094	-0.0002	0.0191	-0.0029	-0.0175	0.0116	0.0000	-0.0096	0.0097
Model 2	0.0079	-0.0022	0.0181	**0.0107**	**0.0012**	**0.0202**	-0.0046	-0.0189	0.0096	-0.0004	-0.0098	0.0090
Model 3	0.0055	-0.0052	0.0161	**0.0105**	**0.0003**	**0.0206**	-0.0080	-0.0219	0.0060	0.0018	-0.0080	0.0117
Model 4	0.0043	-0.0074	0.0160	0.0099	-0.0002	0.0201	-0.0077	-0.0216	0.0062	0.0016	-0.0082	0.0115
Recovery from Mental Stress[2]												
Model 1	0.0005	-0.0080	0.0089	-0.0025	-0.0109	0.0058	0.0038	-0.0071	0.0147	0.0049	-0.0031	0.0129
Model 2	-0.0003	-0.0086	0.0080	-0.0034	-0.0115	0.0047	0.0033	-0.0074	0.0141	0.0055	-0.0024	0.0133
Model 3	0.0013	-0.0074	0.0100	-0.0022	-0.0107	0.0063	0.0047	-0.0060	0.0155	0.0039	-0.0043	0.0122
Model 4	0.0033	-0.0062	0.0128	-0.0016	-0.0101	0.0070	0.0042	-0.0066	0.0151	0.0044	-0.0039	0.0126
Reactivity to Orthostatic Stress[3]												
Model 1	0.0077	-0.0048	0.0202	0.0033	-0.0087	0.0154	-0.0061	-0.0214	0.0092	0.0084	-0.0040	0.0208
Model 2	0.0072	-0.0052	0.0195	0.0038	-0.0088	0.0165	-0.0090	-0.0251	0.0071	0.0083	-0.0042	0.0207
Model 3	0.0094	-0.0040	0.0227	0.0054	-0.0079	0.0186	-0.0111	-0.0284	0.0061	0.0083	-0.0051	0.0216
Model 4	0.0099	-0.0050	0.0248	0.0052	-0.0082	0.0186	-0.0111	-0.0284	0.0062	0.0082	-0.0051	0.0216

*Short sleep was categorized as <6 hours vs. longer ≥6 hours (reference) or as <7 hours vs. longer ≥7 hours (reference)

[1] Reactivity to Mental Stress by Sleep Duration = HR at mental stress - HR at baseline
[2] Recovery from Mental Stress by Sleep Duration = HR at recovery - HR at mental stress
[3] Reactivity to Orthostatic Stress by Sleep Duration = HR at orthostatic stress - HR at baseline

Note: A more positive coefficient for the reactivity associated with shorter sleep means a greater HR increase in response to the stressor (greater reactivity); a more negative coefficient for recovery associated with shorter sleep means a greater HR recovery from the stressor (refer to Figure 2, Supplemental Table 2 & Supplemental Figure 1)

Model 1 = age, gender, race/ethnicity, income-wealth index. Model 2 = model 1 plus body mass index, smoking, alcohol consumption, medications (antihypertensive, antidepressants, sympatho-mimetic medications and medication for sleep and mood), diabetes and sleep efficiency. Model 3 = model 2 plus apnea. Model 4 = model 3 plus naps (hr/day)

Supplemental Table D-6 Mean differences in log transformed HF-HRV (log(msec^2)) at baseline and mean differences in responses to challenge by sleep duration, sleep efficiency and insomnia symptoms (N = 3450 observations, 527 persons)

	Sleep Duration*				**Sleep Efficiency**			**Insomnia Symptoms**		
	(< 6 hrs vs. ≥ 6 hrs)			**(< 7 hrs vs. ≥ 7 hrs)**			**low vs. high**			**Insomnia vs. non-insomnia**		
	Estimate	95% C.I.		Estimate	95% C.I.		Estimate	95% C.I.		Estimate	95% C.I.	
Baseline												
Model 1	0.05	-0.23,	0.32	**-0.33**	**-0.60,**	**-0.07**	**-0.52**	**-1.01,**	**-0.04**	-0.03	-0.29,	0.23
Model 2	0.06	-0.20,	0.33	**-0.28**	**-0.54,**	**-0.03**	-0.48	-0.96,	0.00	-0.03	-0.29,	0.22
Model 3	0.00	-0.28,	0.28	**-0.30**	**-0.57,**	**-0.03**	**-0.60**	**-1.04,**	**-0.15**	-0.01	-0.27,	0.25
Model 4	-0.01	-0.31,	0.28	**-0.31**	**-0.58,**	**-0.04**	**-0.58**	**-1.03,**	**-0.14**	-0.02	-0.27,	0.23
Reactivity to Mental Stress[1]												
Model 1	-0.08	-0.23,	0.07	-0.09	-0.24,	0.06	-0.05	-0.27,	0.17	-0.14	-0.29,	0.01
Model 2	-0.09	-0.24,	0.06	-0.09	-0.25,	0.06	-0.01	-0.24,	0.21	-0.12	-0.27,	0.03
Model 3	-0.05	-0.21,	0.11	-0.10	-0.26,	0.07	0.12	-0.09,	0.33	-0.13	-0.30,	0.03
Model 4	-0.09	-0.26,	0.09	-0.11	-0.28,	0.05	0.13	-0.08,	0.34	-0.15	-0.31,	0.02
Recovery from Mental Stress[2]												
Model 1	0.04	-0.09,	0.17	**0.14**	**0.01,**	**0.26**	0.05	-0.11,	0.21	0.10	-0.02,	0.23
Model 2	0.05	-0.07,	0.18	**0.15**	**0.02,**	**0.27**	0.01	-0.16,	0.18	0.08	-0.04,	0.20
Model 3	0.02	-0.12,	0.15	**0.14**	**0.02,**	**0.27**	-0.03	-0.21,	0.15	0.08	-0.05,	0.21
Model 4	0.02	-0.13,	0.17	**0.15**	**0.02,**	**0.28**	-0.03	-0.21,	0.14	0.09	-0.04,	0.22
Reactivity to Orthostatic Stress[3]												
Model 1	-0.05	-0.24,	0.14	0.05	-0.15,	0.25	-0.03	-0.24,	0.17	**-0.20**	**-0.41,**	**-0.00**
Model 2	-0.04	-0.23,	0.14	0.06	-0.15,	0.26	0.00	-0.22,	0.22	-0.20	-0.40,	0.01
Model 3	-0.10	-0.30,	0.10	0.04	-0.16,	0.25	0.08	-0.15,	0.31	-0.21	-0.44,	0.01
Model 4	-0.16	-0.37,	0.06	0.02	-0.18,	0.23	0.10	-0.14,	0.33	**-0.22**	**-0.44,**	**-0.00**

*Short sleep was categorized as <6 hours vs. longer ≥6 hours (reference) or as <7 hours vs. longer ≥7 hours (reference)

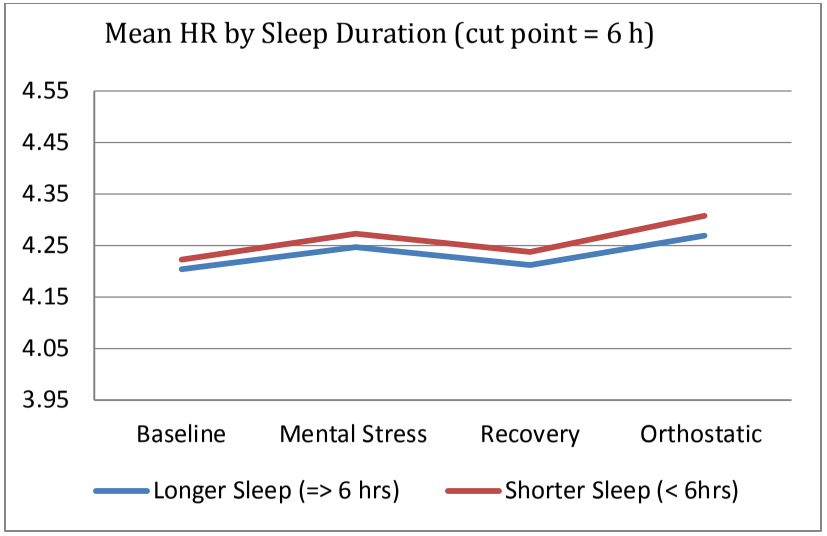
[1] Reactivity to Mental Stress by Sleep Duration = HF-HRV at mental stress - HF-HRV at baseline

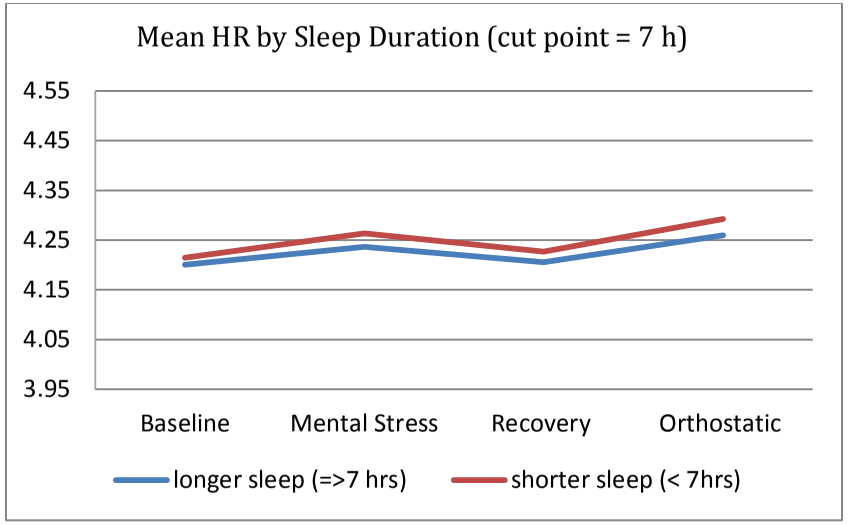
[2] Recovery from Mental Stress by Sleep Duration = HF-HRV at recovery - HF-HRV at mental stress

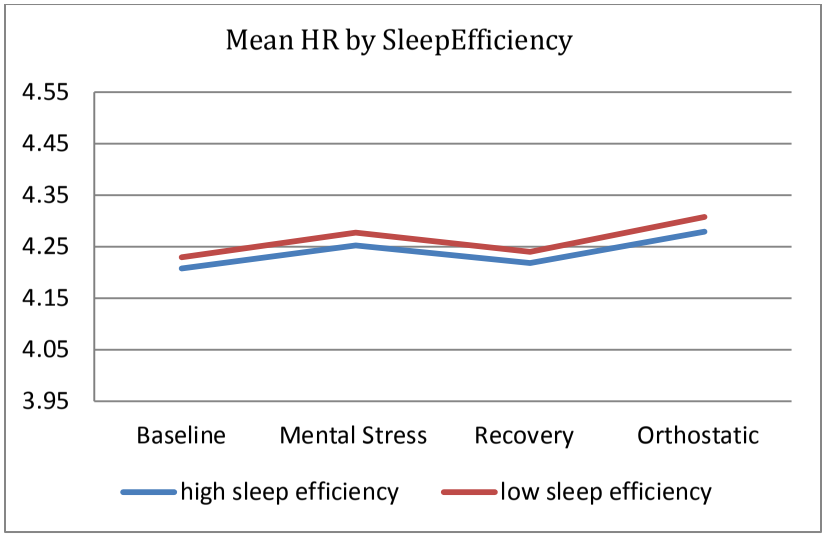
[3] Reactivity to Orthostatic Stress by Sleep Duration = HF-HRV at orthostatic stress - HF-HRV at baseline

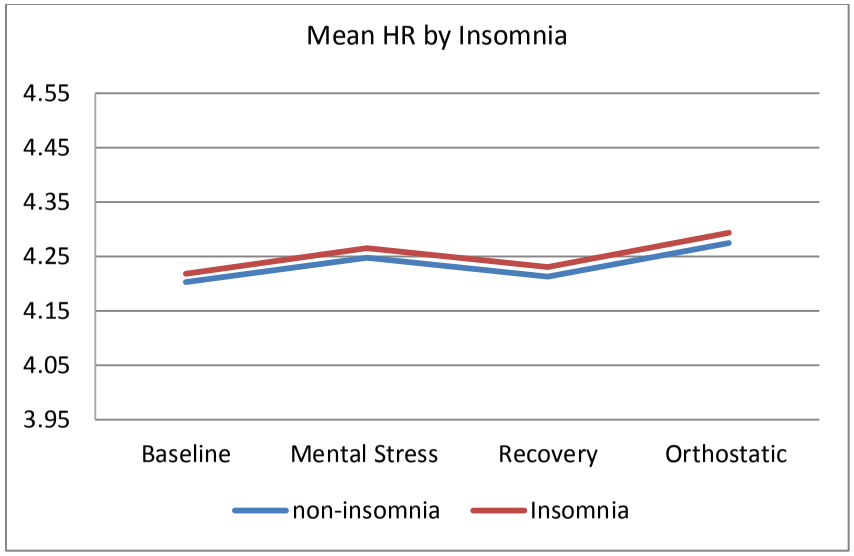
Note: A more negative coefficient for the reactivity associated with shorter sleep means a greater HF-HRV reduction in response to the stressor, a more positive coefficient for recovery associated with shorter sleep means a greater increase in HF-HRV during recovery (Refer to Figure 2, Supplemental Table 3 & Supplemental Figure 2). Model 1 = age, gender, race/ethnicity, income-wealth index. Model 2 = model 1 plus body mass index, smoking, alcohol consumption, medications (antihypertensive, antidepressants, sympatho-mimetic medications and medication for sleep and mood), diabetes and sleep efficiency. Model 3 = model 2 plus apnea. Model 4 = model 3 plus naps (hr/day)

Supplemental Figure D-1 Mean log-transformed heart rate (HR) (log beats/min) during the stress challenge protocol, Mesa Stress

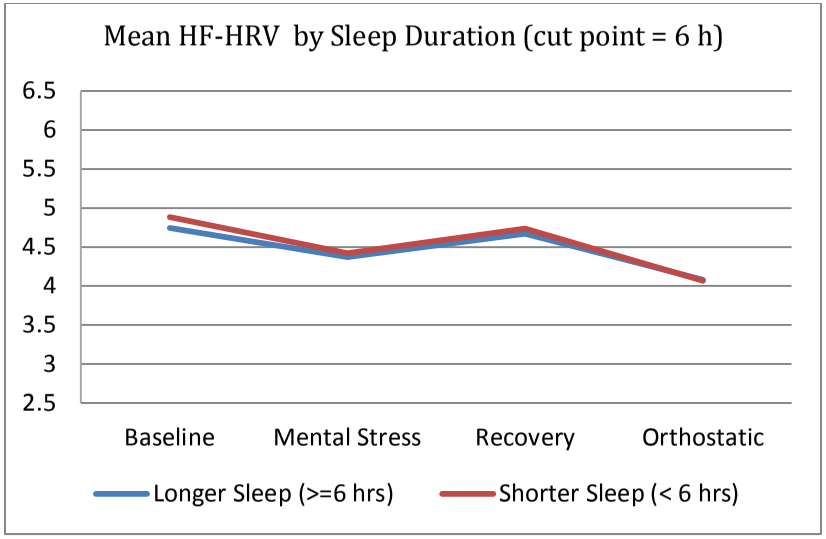


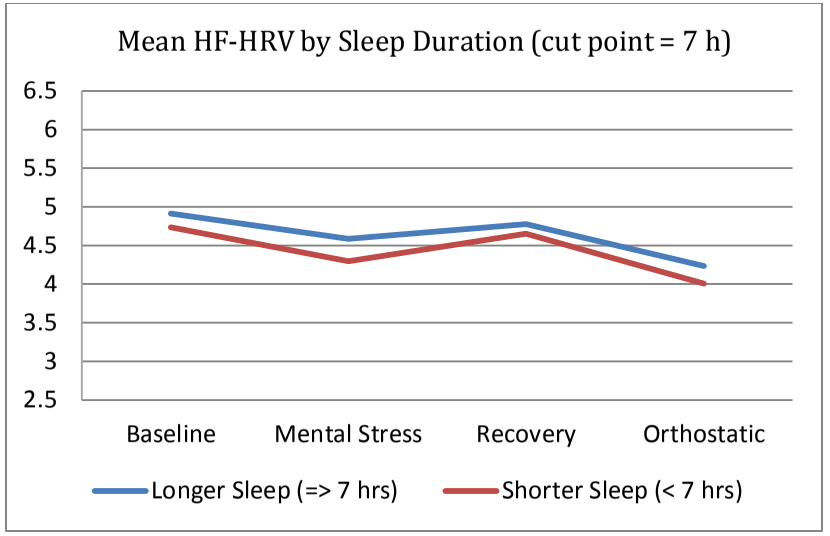


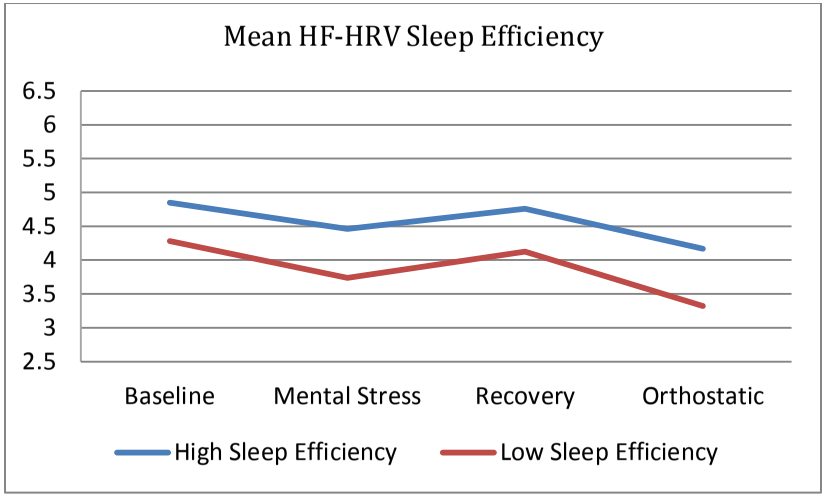


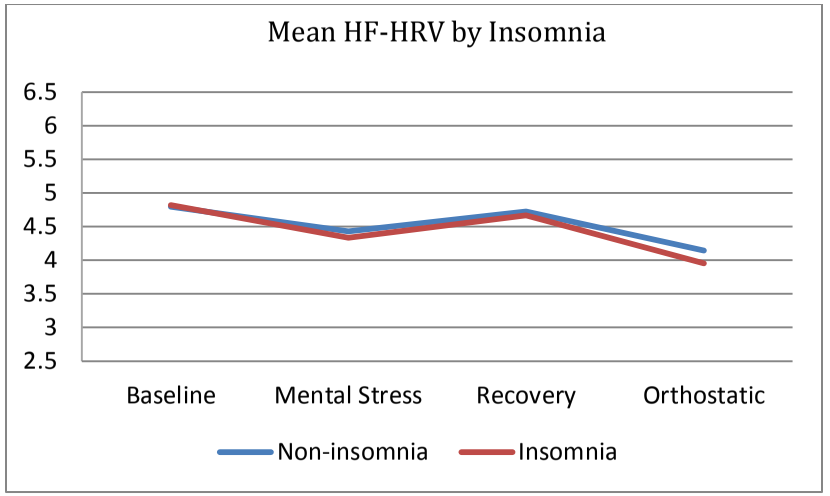


Supplemental Figure D-2 Mean log-transformed high frequency HRV (log msec^2) during the stress challenge protocol, Mesa Stress

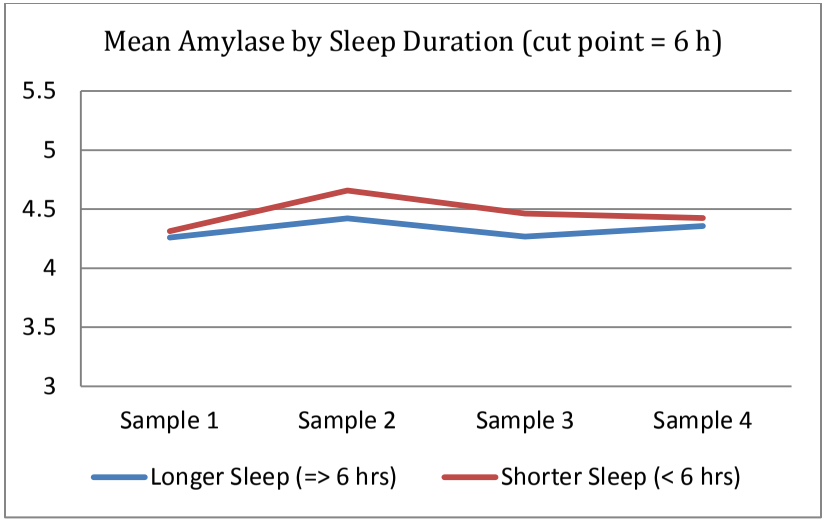


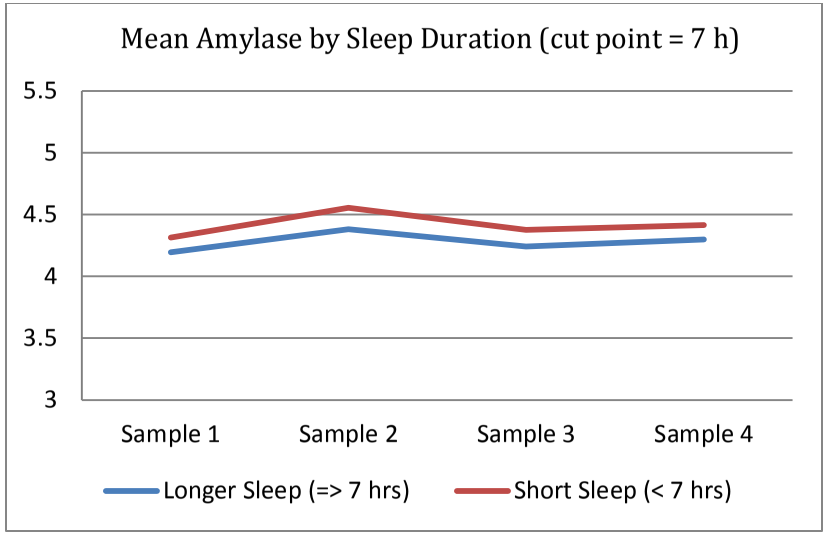


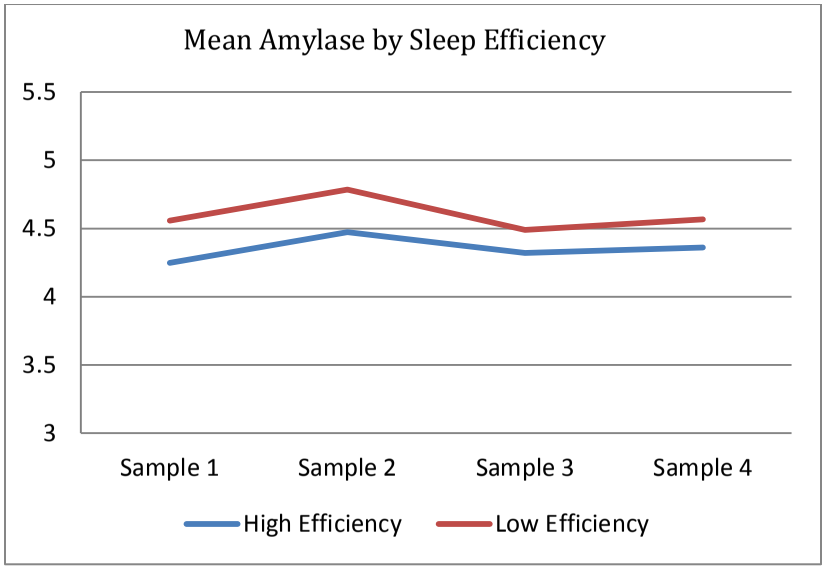


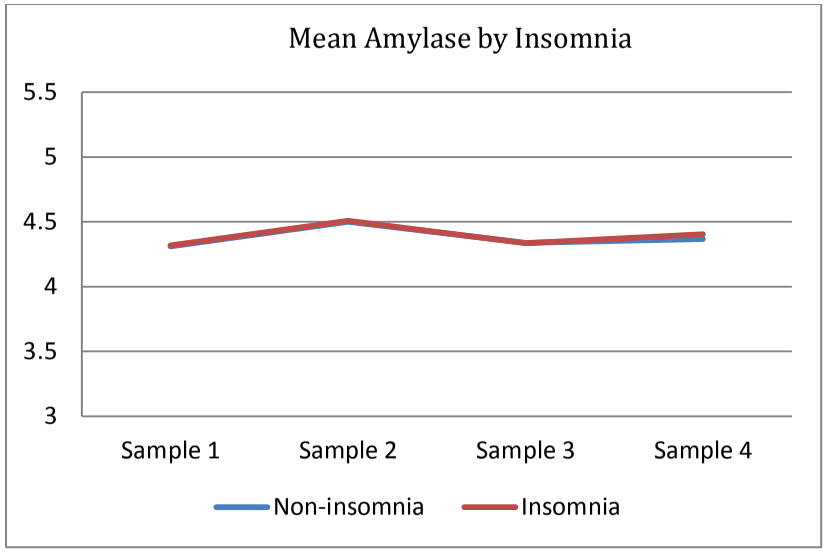


Supplemental Figure D-3 Mean log-transformed amylase (log U/mL) during the stress challenge protocol, MESA Stress

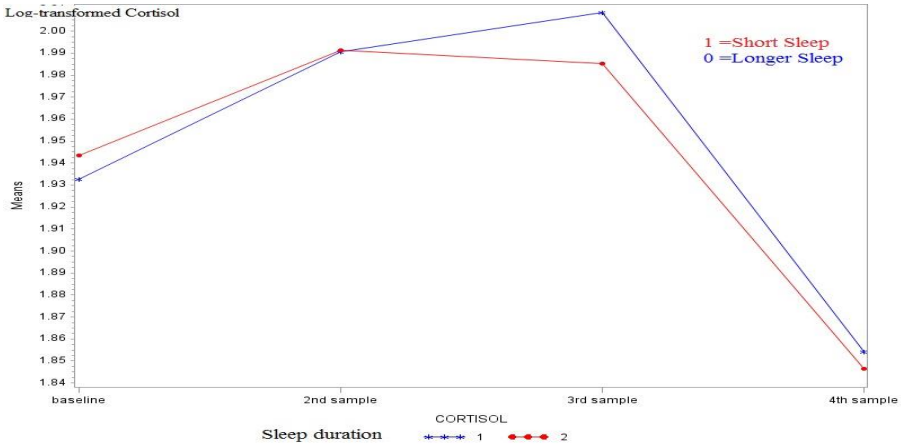


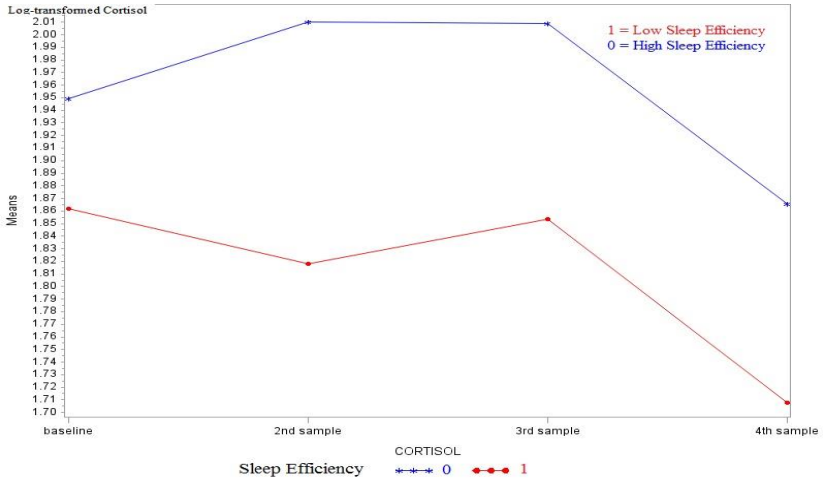


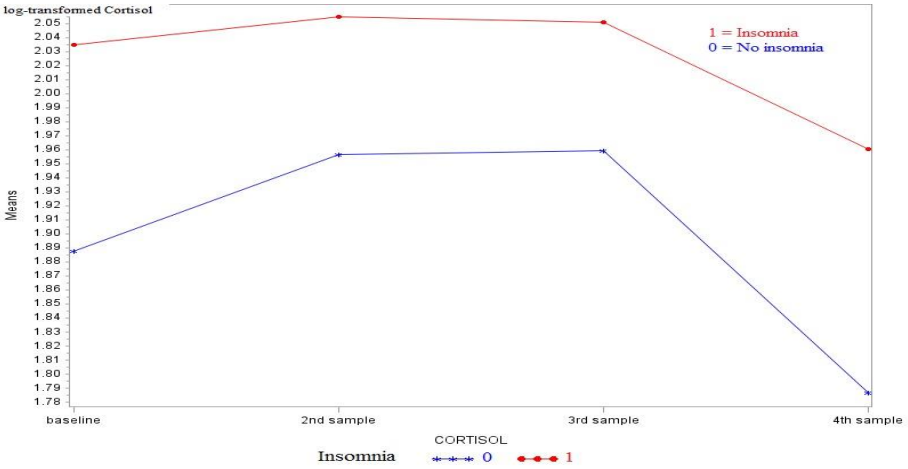




Supplemental Figure D-4 Mean log-transformed cortisol (log nmol/L) during the stress challenge protocol by sleep duration, sleep efficiency an insomnia, MESA Stress

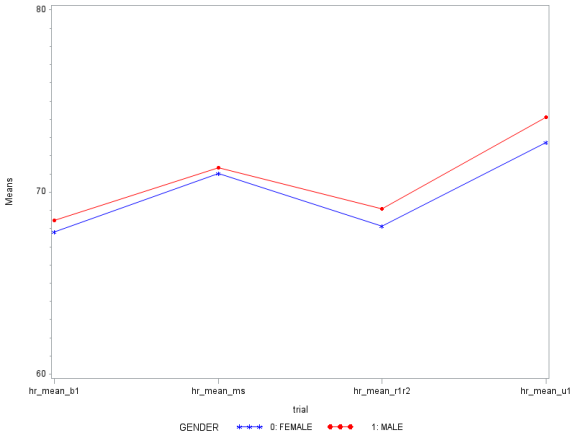




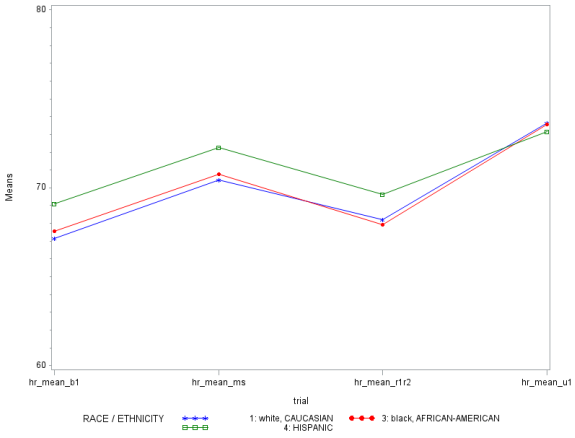


Supplemental Figure D-5 Mean log-transformed cortisol (log nmol/L) during the stress challenge protocol by demographics, MESA Stress

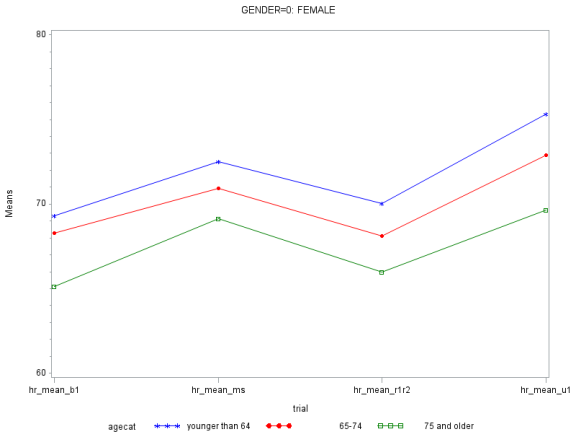
Heart Rate by Gender



Heart Rate by Race/Ethnicity



Heart Rate by Age - Females



Heart Rate by Age - Males